REVIEWS OF PURE AND APPLIED CHEMISTRY

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The Terpenoid Bitter Principles

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1 INTRODUCTION

Many classes of chemical compounds contain bitter-tasting substances; for example, most of the alkaloids, several coumarins and terpenoids and many other miscellaneous substances have bitter tastes. Only the terpenoid bitter substances will be dealt with in this review.

In view of the diversity of chemical types of bitter substances it can be inferred that there is no close correlation between bitterness and chemical structure. In the terpenoid bitter compounds, however, the lactone group is very commonly present although it is absent in the cucurbitacin bitter principles (see section 5.0.1).

1.1 *Physiological activity*

A great many curative effects have been attributed to bitter substances and they have long been used in many pharmaceutical preparations. Some of these claims are erroneous, others have been substantiated.

Bitter substances generally stimulate the secretion of gastric juices and because of this bitters have been used in medicine to increase the appetite and aid the digestion. Gentian, Quassia and Columba are the only bitters that are largely used and they are alleged to have no other physiological activity. Many of the terpenoid bitter substances have more far-reaching physiological effects. Thus, some may cause paralysis of the heart and other muscles, some have been used as hypnotics, anthelmintics and respiratory stimulants and recently, the cucurbitacins have been shown to possess anti-tumor activity[1].

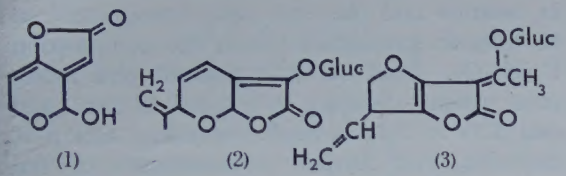
2 MONOTERPENOID BITTER SUBSTANCES

This is the smallest group of bitter substances and the only one whose members are glycosides.

2.0.1 *Gentiopicrin*

Gentiopicrin, a glucoside occurring in *Gentiana lutea*, has been known since 1862 and although it has been investigated by several workers[2, 3, 4] its structure is still unknown. This is undoubtedly due to its instability under acidic or basic conditions and in this and other respects it resembles patulin (1). Several structures (2)[2], (3)[3], (4)[3], (5)[4], have been ad-

vanced but none is compatible with all of the chemical and spectroscopic evidence.



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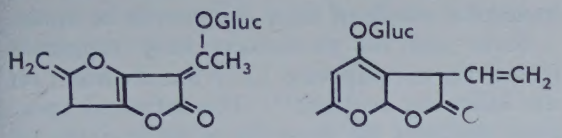

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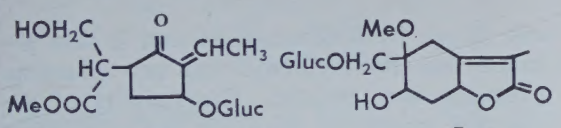
2.0.2 *Loganin*

The *Strychnos* species is probably best known as a source of strychnine and other alkaloids. *Strychnos nux vomica* and *Strychnos lucida,* however, also contain a bitter principle known as loganin.

Two groups of workers have proposed alternative structures (6)[5] and (7)[6, 7] for loganin and in fact, it has been suggested[8] that they

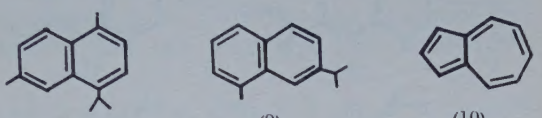


(6) (7)

were working with different compounds. The available evidence does not strongly support this suggestion and indeed the reactions and spectral properties attributed to (7) are more consistent with structure (6). On the basis of the ultra-violet and infra-red spectra and the formation of a 2,4-dinitrophenylhydrazone, Birch and Smith[5] proposed structure (6). Merz and Lehmann[7] made a 2,4-dinitrophenylhydrazone with practically the same melting point from their loganin. The CH_3–CH= grouping followed from the Kuhn-Roth determination and the results of ozonolysis[5]. Mild treatment with alkali afforded methanol and this, in conjunction with the infra-red spectrum, indicated the presence of a methyl ester grouping[5]. Since loganin is not a methylated β-keto-acid, structure (6) was tentatively proposed[6], it being assumed that loganin is isoprenoid.

3 SESQUITERPENOID BITTER SUBSTANCES

This constitutes by far the largest group of bitter terpenoid substances. The sesquiterpenoids can be classified into groups of related compounds according to the nature of their carbon skeletons. Thus there are: (a) aliphatic compounds, (b) substances which possess 9



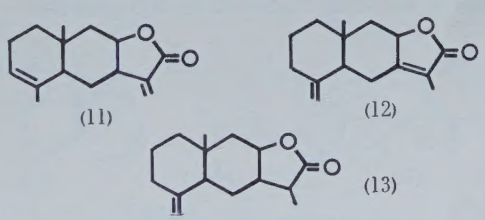
(8) (9) (10)

to 11 membered rings, and those sesquiterpenoids which on dehydrogenation yield (c) cadalene (8), (d) eudalene (9) and (e) azulene (10) derivatives. All of these groups except the aliphatics contain bitter principles.

3.1 *Eudalene-type substances*

3.1.1 *Alantolactone*

Three bitter substances occur in the essential oil of the roots of *Inula helenium;* they are alantolactone (11), isoalantolactone (12), and dihydroisoalantolactone (13). The anthelmin-



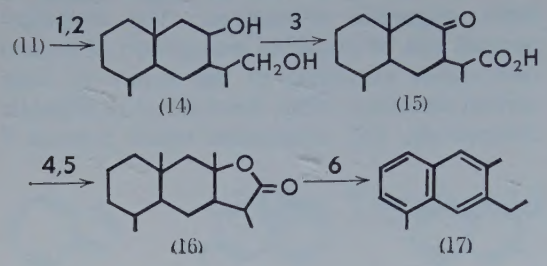
(11) (12) (13)

tic properties of the alantroot are attributed to the presence of these compounds. All three are lactones and alantolactone and isoalantolactone possess two double bonds.

Selenium dehydrogenation of suitable derivatives gave 1-methyl-7-isopropyl naphthalene thereby revealing the carbon skeleton[9]. The position of attachment of the alkyl oxygen of the lactone ring was proved[10] as shown (see (11) to (17)).

One of the two double bonds of alantolactone and isoalantolactone was shown to be reducible by sodium amalgam and hence is α,β- to the lactone carbonyl group. The position of the second double bond was found by ozonolysis

of the dihydro derivatives of these compounds[11]. Sorm and his colleagues[12] have



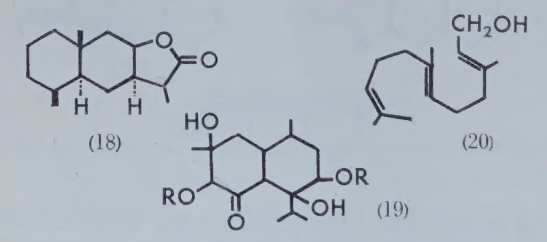
Reagents 1, H_2; 2, $LiAlH_4$; 3, CrO_3; 4, CH_2N_2; 5, *MeMgI*; 6, *Se*.

shown that the absolute configuration of tetrahydroalantolactone is that in (18).

3.2 *Cadalene-type compounds*

3.2.1 *Laserpitin*

Laserpitin, the bitter principle from *Laserpitium latifolium* has recently been shown to have the structure (19, R = ·CO·CMe:CHCH$_3$)[13]. Although its structure is isoprenoid it is not derived directly from the cyclization of farnesol (20). However, it may be considered to be a member of the cadalene group in which a methyl group has migrated during the final stages of its biosynthesis.



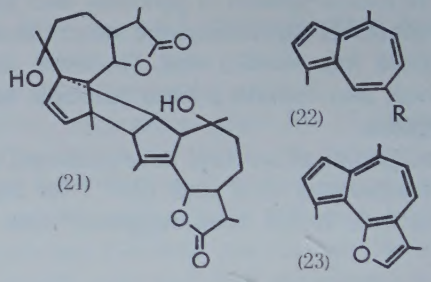
Mild hydrolysis gave laserol (19, R = H) which was converted into 1,7-dimethyl-4-isopropyl napthalene. Oxidative degradation of laserpitin and its derivatives and dehydration experiments established the location of the functional groups.

3.3 *Perhydroazulene derivatives*

3.3.1 *Absinthin and anabsinthin*

These compounds are the bitter principles of *Artemesia absinthium*. On the basis of a cryoscopic determination of molecular weight in tertiary butanol, they were once thought to be guaianolides with molecular formulae $C_{15}H_{20}O_3$ and $C_{15}H_{20}O_3 \cdot \frac{1}{2}H_2O$, respectively[14]. More recently their molecular weights have been determined by isothermal distillation in acetone and they are now considered[15] to be dimeric guaianolides with the composition $C_{30}H_{40}O_6$. That the two methods give molecular weights differing by a factor of two (270 and 520 ± 50) is most interesting and it is desirable that further measurements of the molecular weight of these compounds be made.

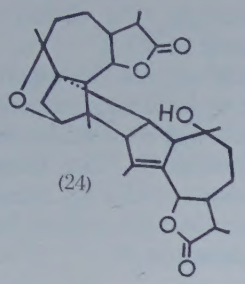
Sorm and his co-workers have suggested tentatively the structure (21) for absinthin on the following evidence[15]. The infra-red spectrum indicates the presence of at least one of each of the following functional groups: a γ-lactone, an ethylenic linkage and a hydroxyl group. The presence of two double bonds



was shown by hydrogenation and it was assumed that two hydroxyl and two γ-lactone groupings were present. The stability of the hydroxyl groups to oxidation and acetylation indicates that they are tertiary. Selenium dehydrogenation of suitable derivatives gave 12-hydroxyguaiazulene, chamazulene (22, R = Et), guaiazulene (22, R = *iso*Pr), and artemazulene (23). The structure (21) was proposed on the assumption that absinthin might be formed by a Diels-Alder addition of the appropriate dienes.

The infra-red spectrum of anabsinthin indicates the presence of at least one lactone group and the absence of hydroxyl groups and double bonds. A hydroxyl group was presumed to be present from the results of an active hydrogen determination and was confirmed by the dehydration of anabsinthin with formic acid to give a conjugated diene. This also indicated the presence of a double bond in anabsinthin. Absinthin is converted into anabsinthin when it is dissolved in ether containing traces of hydrochloric acid, or when it is heated above its melting point or crystallized from high boil-

ing solvents. This transformation was envisaged as occurring by the addition of one of the

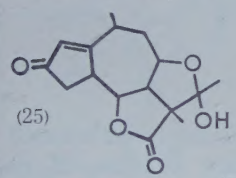


(24)

hydroxyl groups onto a double bond to form an oxide ring as shown in (24).

3.3.2 *Tenulin and isotenulin*

Tenulin (25) and isotenulin (26) are bitter principles of *Helenium tenuifolium, H. elegans, H. badium* and *H. montanum*. The formation of chamazulene (22, R = Et) and linderazulene (27) in selenium dehydrogenation ex-



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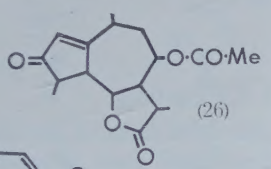

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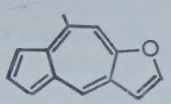
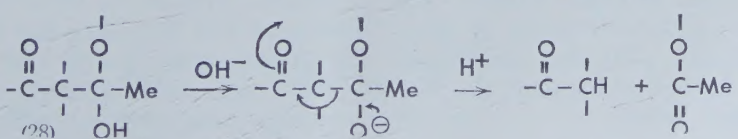


(27)

periments established the nature of their carbon skeletons[16].

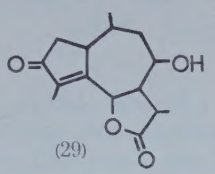
Infra-red and ultra-violet spectra show that tenulin is a γ-lactone possessing an α,β-unsaturated cyclopentenone system and a hydroxyl group. Mild treatment with alkali converts tenulin to isotenulin[17] which is a γ-lactone possessing an α,β-unsaturated cyclopentenone system and an acetate grouping. The fact that tenulin gives no acetic acid on acid hydrolysis whereas isotenulin provides one mole of acetic acid under the same conditions was explained[16] by postulating a masked acetate grouping (as in 28) which is cleaved by alkali to isotenulin as follows:



(28)

The carbonyl group in (28) has been shown to be that of the γ-lactone[16].

Tenulin has the cyclopentenone system as in (25) since digestion with alkali converts it into deacetylneotenulin (29) which yields acetic acid on ozonolysis whereas tenulin does not. During this transformation the lactone ring remains unopened[16]. Because the oxidation product of deacetylneotenulin did not have the ultra-violet spectrum of an ene-1,4-dione and was not reduced by zinc and acetic acid it was formulated as in (30) and the structures for tenulin (25) and isotenulin (26) followed[16].



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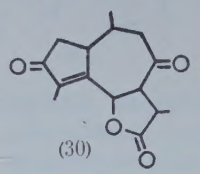
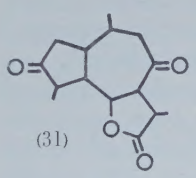


(30)

Conflicting evidence was produced by Herz and his co-workers[18] when they observed that deacetyldihydrodehydroisotenulin (31) was cleaved by alkali to a dicarboxylic acid containing an α,β-unsaturated ketone grouping. They formulated tenulin as (32) and the cleavage product as (33) but this structure for tenulin does not explain the properties of neotenulin.



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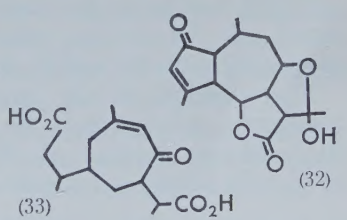

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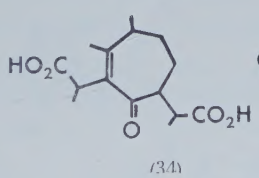

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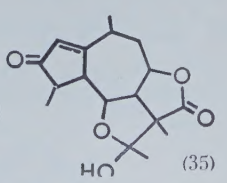


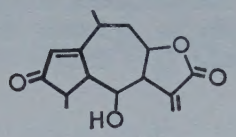
(35)

Alternative structures for the cleavage product (34) and for tenulin (35) were suggested as being more compatible with these observations[19]. However, since the oxidation product of deacetylneotenulin is not an ene-1,4-dione tenulin cannot have structure (35). Herz

and his colleagues have reported other evidence which tends to support the position of the lactone ring as in (25).

3.3.3 *Helenalin and isohelenalin*

Various *Helenium* species contain the bitter substances helenalin (36) and isohelenalin (37). Helenalin is a fish poison and possesses



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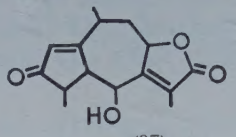
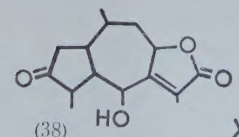


(37)

anthelmintic properties. It also affects the mucous membranes, stomach and intestines and causes paralysis of the heart and other muscles[19].

The presence of a γ-lactone, a secondary hydroxyl group, a $>C=CH_2$ grouping and an α,β-unsaturated cyclopentenone system in helenalin was established by Adams and Herz[20]. Helenalin and isohelenalin were both shown to be α,β-unsaturated γ-lactones and to differ only in the position of this double bond[21]. The location of the α,β-unsaturated ketone grouping was based on quantitative measurements of the nuclear magnetic resonance spectra of helenalin and its derivatives[21].

The position of the secondary hydroxyl group was deduced from the observation that the oxidation product of dihydroisohelenalin (38) is a conjugated ene-dione (39) capable



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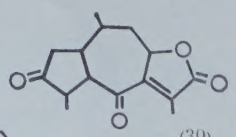

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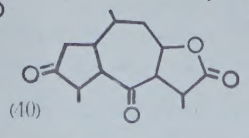


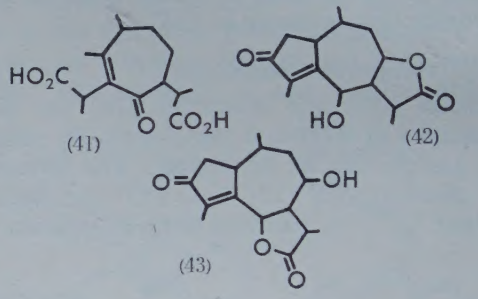
(40)

of being reduced by zinc and acetic acid to tetrahydrodehydrohelenalin (40) which was also obtainable from helenalin (36)[21]. The interesting transformation by alkali of tetrahydrodehydrohelenalin (40) into the dicarboxylic acid (41) further substantiates the position of the hydroxyl group[21].

3.3.4 *Geigerin*

Geigerin (42) is a bitter substance occurring in *Geigeria aspera* Harv., the 'vomiting bush' of South Africa. This plant causes heavy annual losses of sheep and the suspected poisonous principle is a closely related substance, vermeeric acid[22].

The location of the hydroxyl group was demonstrated by oxidizing geigerin to an ene-1,4-dione which was reduced by zinc and acetic acid to a 1,4-dione[23]. The formation of an isomeric γ-lactone (43) by treating geigerin methanesulphonate with a base, fixed the position of the lactone ring[23].



(41) (42) (43)

3.3.5 *Lactucin*

The dried latex of *Lactuca virosa* (wild lettuce) has been used in pharmacy because of its reputation as a hypnotic. This physiological activity has been attributed to the presence of lactucin (44).

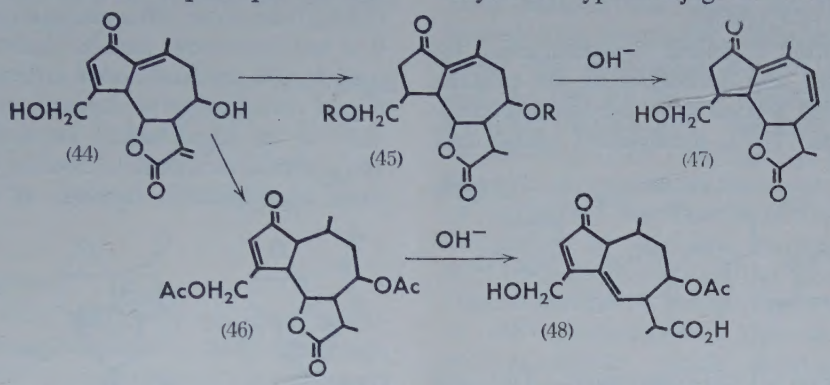
Lactucin is biogenetically interesting because it is the first sesquiterpenoid lactone of the azulene series with a carbonyl group at C(2). This feature was established on the basis of the ultra-violet and infra-red spectra of lactucin and its derivatives[24].

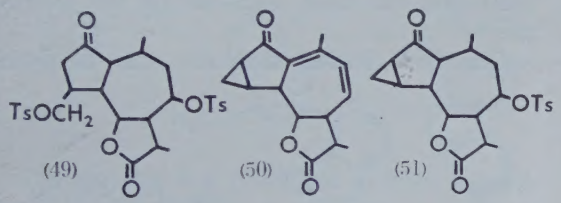
The positions of the secondary hydroxyl group and the alkyl oxygen of the lactone group relative to the cyclopentenone carbonyl group were established by base-catalysed eliminations on the hydrogenation products (45, R = H) and (46) of lactucin[24].

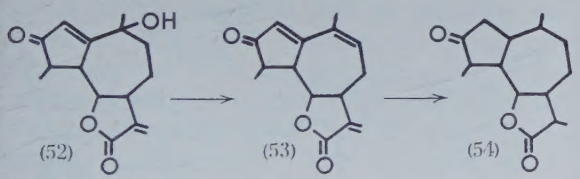
The location of the primary hydroxyl group was elegantly demonstrated in the following manner. Both of the ditosylates of tetrahydrolactucin (45, R = Ts) and hexahydrolactucin (49) when treated with base form derivatives (50) and (51), respectively, containing a cyclopropane ring conjugated with the carbonyl group. The formation of the dienone system in (50) proves the location of the primary hydroxyl group[24].

3.3.6 *Parthenin*

Parthenin (52) is a bitter principle of *Parthenium hysterophorus* L. Its constitution was deduced mainly by converting it into tetrahydroambrosin (54)[25].







3.4 *Compounds possessing ten-membered rings*

3.4.1 *Pyrethrosin*

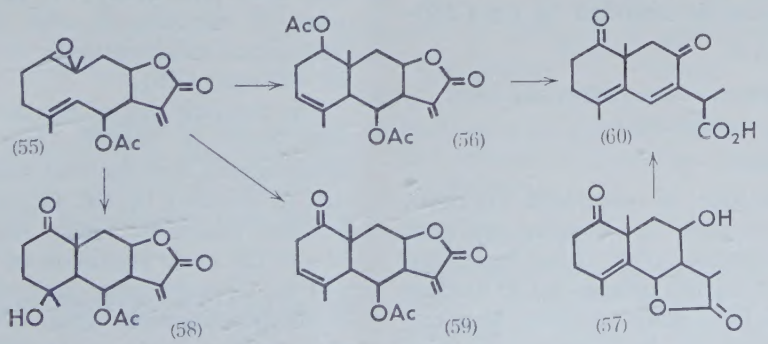
Chrysanthemum cinerariaefolium which is better known as a source of the pyrethrin insecticides also contains a bitter principle pyrethrosin (55). Although pyrethrosin was first isolated[26] in 1891 its structure has only recently been elucidated[27]. It has been shown to possess two double bonds, one of which is a vinylidene type conjugated with a γ-lactone system. An acetoxyl group was detected and an ethereal oxygen was deduced to be present. Pyrethrosin is therefore monocarbocyclic.

The facile cyclization reactions of pyrethrosin provided the main evidence for its structure. Treatment with acetic anhydride and toluene-*p*-sulphonic acid converted pyrethrosin into cyclopyrethrosin acetate (56) which is related to ψ-santonin (57). Cyclization of pyrethrosin also occurs readily under mild oxidative conditions with sodium dichromate and aqueous acetic acid at room temperature. Two products (58) and (59) are obtained, one of which has been related to cyclopyrethrosin acetate (56), Mechanistic considerations of these cyclizations lead to the structure (55) for pyrethrosin.

Pyrethrosin is biogenetically interesting because of its constitution and ease of cyclization. Most of the bicyclic sesquiterpenoids can be constructed theoretically from pyrethrosin by cyclization at different points across its ring.

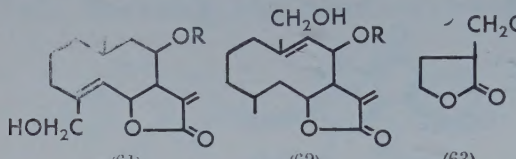
3.4.2 *Cnicin*

Cnicin, the bitter principle of *Cnicus benedictus* L. was once thought to be a guaianolide-type lactone[28]. Recently it has been shown to have



the ten-membered ring represented by structures (61) or (62)[29] in which R = –CO–C-(CH_2OH)=CH–CH_2OH.

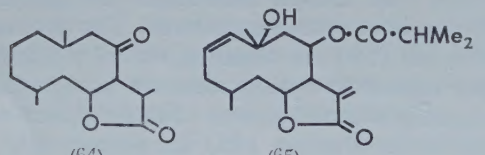
Hydrogenation of cnicin is accompanied by hydrogenolysis, and hydrolysis of the mixture obtained in this process gave 2-hydroxymethyl-γ-butyrolactone (63), methylethyl acetic acid



(61) (62) (63)

and two neutral C(15)-compounds. This and spectral evidence indicated the presence of the grouping –O–CO–C(CH_2OH)=CH–CH_2OH in cnicin[29].

The two neutral C(15)–compounds were found to have a primary and a secondary hydroxyl group. Oxidation of a non-crystalline fraction of the hydrogenation products afforded a keto-lactone (64) which was related to arctiopicrin (65)[30], a sesquiterpenoid lactone isolated from *Arctium minus* Bernh. The precursor of the



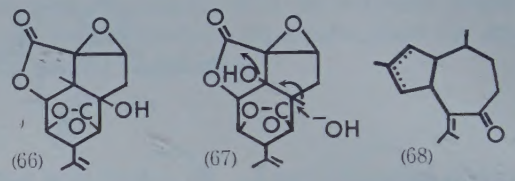
(64) (65)

keto-lactone (64) must have been a hydroxy-lactone which was formed by the hydrogenolysis of the primary hydroxyl group in cnicin. Thus one of the double bonds of cnicin is in an allylic position to the primary hydroxyl group. Oxidation experiments on the hydrogenation product of cnicin demonstrated that the secondary hydroxyl group is esterified by the C(5)-acid[29].

3.5 *Miscellaneous sesquiterpenoid bitter substances*

3.5.1 *Picrotoxinin*

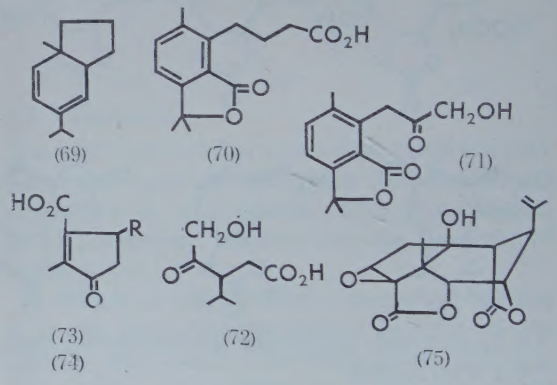
The bitter principle of *Anamirta cocculus, Menispermum cocculus* or *Cocculus indicus* is known as picrotoxinin (66). It has been used medicinally since the last century but its modern usage began in 1933 when its value as a respiratory stimulant in barbiturate poisoning was demonstrated. Picrotoxinin also causes slowing of the heart beat, sweating, a lowering of the body temperature and is toxic when excessive doses are administered[31]. Picrotoxinin differs from most other sesquiterpenoids in that it is not isoprenoid and its skeleton is not derived by the cyclization of farnesol. However, it can conceivably be formed by a 1,2-methyl shift in the hypothetical isoprenoid precursor (67) which, incidentally, has the same arrangement of isoprenoid residues as zierone (68).



(66) (67) (68)

Proof of the skeletal structure[32] came from the degradation of picrotoxinin to picrotoxadiene (69) and the total synthesis of this derivative[33].

Conroy[32] showed that the acid catalysed

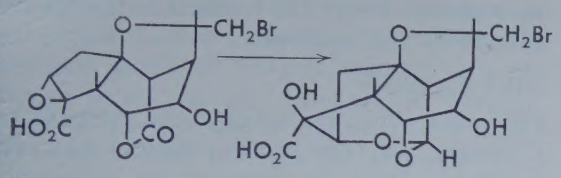


(69) (70) (71) (73) (74) (72) (75)

aromatization of picrotoxinin to picrotic acid (70) and picrotonol (71)[34], and the formation of compounds with the structures (72), (73, R = OH) and (74, R = H) by treatment of picrotoxinin with alkali[35], can be rationalized mechanistically if picrotoxinin has the structure (66).

The epoxide ring of picrotoxinin is remarkably stable to acids[36]. The structure (75) demonstrates that the rear-side of the epoxide ring is shielded by the lactone system which prevents attack by acids. Another manifestation of the close proximity of the epoxide ring and the lactone group was obtained when β-bromopicrotoxininic acid (76) was treated with sodium borohydride. Dihydro-β-bromo-picrotoxininic acid (77) was formed, presumably by a concerted acceptance of a hydride ion

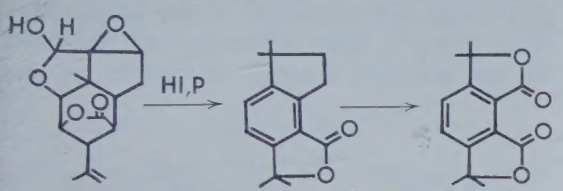
by the carbonyl group and the simultaneous attack of the carbonyl oxygen on the rear-side of the epoxide ring[36].



(76) (77)

3.5.2 *Coriamyrtin*

Coriamyrtin (78) along with tutin is a constituent of *Coriaria japonica*[37]. Both these substances resemble picrotoxinin in their chemical and physiological properties.



(78) (79) (80)

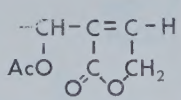
Coriamyrtin was presumed to have a hemi-acetal grouping since it possesses a hydroxyl group and is converted into an aldehyde on treatment with acid[37]. Evidence for the carbon skeleton came from the degradation of coriamyrtin to coriaria lactone (79) and thence to coriaria dilactone (80)[37] which was subsequently synthesized[38]. The migration of a methyl group was assumed to occur under the conditions used. The location of the lactone, hemi-acetal and epoxide rings was based entirely on the fact that coriamyrtin and picrotoxinin have similar physiological and chemical properties.

4 DITERPENOID BITTER SUBSTANCES

4.0.1 *Andrographolide*

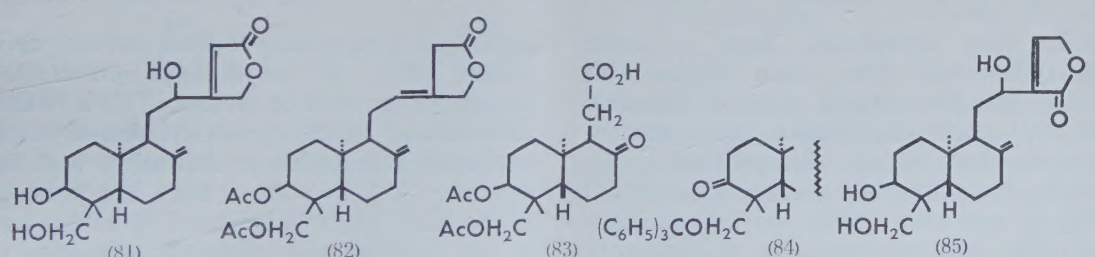
Andrographolide, the bitter principle of *Andrographis paniculata* Nees, has been formulated by Cava and Weinstein[39] as shown in (81). They showed that when the triacetate was treated with aluminium amalgam, an acetoxy group was eliminated and the absorption at 223 mμ due to the α,β-unsaturated γ-lactone grouping disappeared. The diacetyl derivative so obtained (82) and triacetyl andrographolide both give the same diacetyl-keto-acid (83) on oxidation. The formation of 1,5-dimethyl-2-naphthol by selenium dehydrogenation of this keto-acid indicates the position of the original methylene group. Treatment with acid of the monotrityl-keto derivative (84) of andrographolide yields formaldehyde and proves the relationship between the two remaining hydroxyl groups.

Chan, Haynes, and Johnson[40] propose the structure (85) for andrographolide on the grounds that the low infra-red absorption frequency (1725 cm^{-1}) of the carbonyl group in the α,β-unsaturated γ-lactone is due to hydrogen bonding with the adjacent hydroxyl group. Their interpretation of the NMR spectrum of andrographolide triacetate suggested partial structure (85a).



(85a)

They also consider that desoxyandrographolide diacetate is an α,β-unsaturated γ-lactone because of its infra-red and ultra-violet spectra and because it gives the colour test for such a compound.

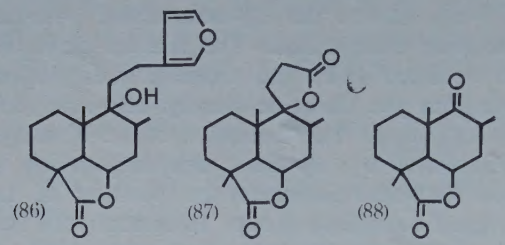


(81) (82) (83) (84) (85)

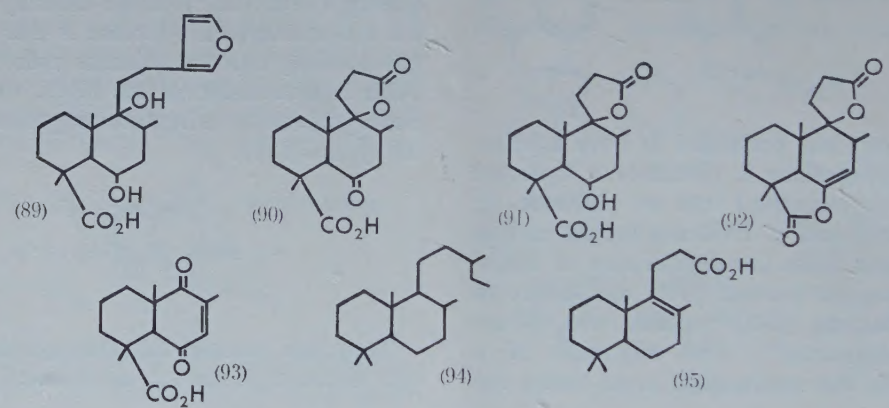
4.0.2 *Marrubiin*

Marrubiin (86) is the bitter principle of horehound (*Marrubiin vulgare* L.). It possesses a γ-lactone grouping, a tertiary alcohol function, an inert oxygen, and has been shown by selenium dehydrogenation to contain a bicyclic carbon skeleton[41]. A furan ring was shown to be

present by a comparison of the infra-red and ultra-violet spectra of marrubiin and of furan and its derivatives[42]. Further evidence for this



grouping was obtained by oxidation experiments in which the furan ring was removed and a dilactone (87) was formed. This suggested the position of the hydroxyl group relative to the furan ring. Dehydration of marrubiin followed by ozonolysis yields a keto-lactone (88)



which, when hydrolysed, gives a stable hydroxy-keto-acid. This, when coupled with the fact that the carboxyl group of marrubic acid (89) resists esterification and is decarboxylated when heated with sulphuric acid, indicates the position of the lactone carbonyl group of marrubiin. Since the lactonic-keto-acid (90) obtained from the oxidation of marrubic acid (89) or of the lactonic-hydroxy-acid (91) could be cyclized to an α,β-unsaturated γ-lactone (92), the position of attachment of the lactone ring of marrubiin must be as indicated. An α,β-unsaturated γ-lactone involving C(7) (naphthalene numbering) is sterically impossible. Other evidence[43] was obtained by oxidizing the keto-hydroxy-acid from (88) to the conjugated ene-1,4-dione (93).

The structure of marrubiin had initially been based on the assumption that its skeleton was as given in (94) although no direct proof for this had been obtained[42]. Eventually, however, the enol-lactone (92) was converted into an unsaturated acid[44] (95) which is a degradation product of ambrein.

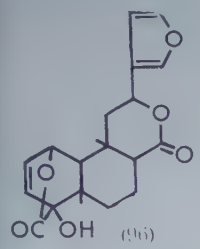
4.0.3 *Columbin*

Columbin (96), the major bitter principle of Columbo root (*Jatrorrhiza palmata*) was first isolated in 1830. Its chemistry was extensively investigated by Feist[45] and Wessely[46] and their co-workers during 1935-1938 but its structure has only recently been elucidated[47].

Columbin is a hydroxy-dilactone which is isomerized to isocolumbin on mild treatment with alkali. This is interpreted as an epimerization at the carbon atom α- to the lactone carbonyl group[47]. Since columbin and isocolumbin but not their dehydro derivatives are decarboxylated on melting to form carbonyl derivatives (97), an α-hydroxy-β,γ-unsaturated lactone system must be present. This is further substantiated by the decarboxylation of acetyl or methyl isocolumbin to derivatives with the structure (98), R = Ac or Me). The presence of the furan ring was indicated by comparison of the spectral and chemical properties of columbin and marrubiin (96). Confirmation was obtained when ozonolysis of dihydrocolumbin gave a C(17)-acid as the major product.

The relationship of the furan ring to the second lactone system was deduced from the fact that this lactone undergoes hydrogenolysis under vigorous hydrogenation conditions. The location of these functional groups in the columbin molecule resulted from a series of dehydrogenation experiments. Wolff–Kishner

reduction of decarboxyoctahydrocolumbinic acid (99) followed by selenium dehydrogenation gives 1-methyl-2-naphthoic acid (100) whose carboxyl group defines the position of the second lactone carbonyl group. When the carbonyl group of (99) is reduced to a hydroxyl group and this compound is dehydrogenated, 1,5-dimethyl-2-naphthoic acid (101) is formed. This involves the migration of a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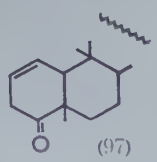
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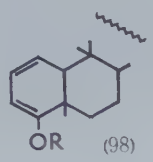
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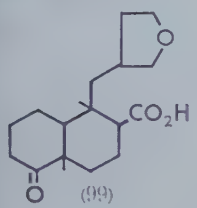
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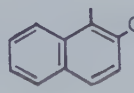
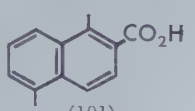
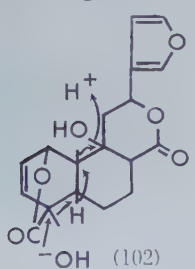

(100)


(101)

tertiary methyl group to the position of attachment of the hydroxyl group. Hence the second lactone carbonyl and the hydroxyl group are attached at C(5) (naphthalene numbering) with a tertiary methyl group adjacent. The other angular methyl group was located as in (96) to account better for the formation of various degradation products.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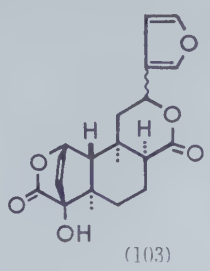
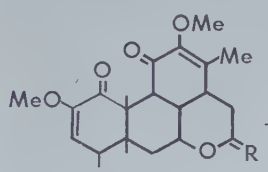

(103)

The biogenesis of columbin has been envisaged as shown in (102) where a normal diterpenoid type compound undergoes the appropriate methyl migrations[47]. Speculations on the stereochemistry of columbin based on the rotatory dispersion curve of decarboxyoctahydrocolumbinic acid (99) and reactions of columbin lead to the structure (103)[48].

4.0.4 *Quassin and neoquassin*

Quassin (104, R = O) and neoquassin (104, R = –H,–OH) are the bitter principles of *Quassia amara* L. Although the chemistry of these substances has been extensively investigated over a number of years[49, 50, 51], their structures have only recently been elucidated[51a].

Earlier work[51] established that quassin, $C_{20}H_{22}O_4(OMe)_2$, is a δ-lactone and neoquassin is the corresponding hemiacetal and that both possess a methylated diosphenol grouping, a methoxyl group, an α,β-unsaturated ketone system, and a perhydrophenanthrene skeleton. Ultra-violet and NMR spectroscopy and deuterium exchange experiments indicated the presence and nature of two diosphenol groupings whose relative positions were demonstrated in the following way[51a]. Norquassinic acid (105), when treated with acetic anhydride and sodium acetate yields a neutral acetate[51], now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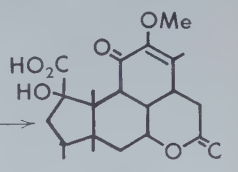
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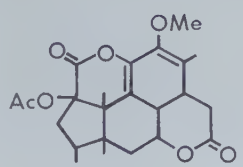
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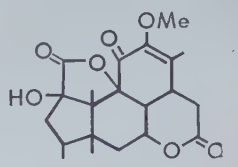
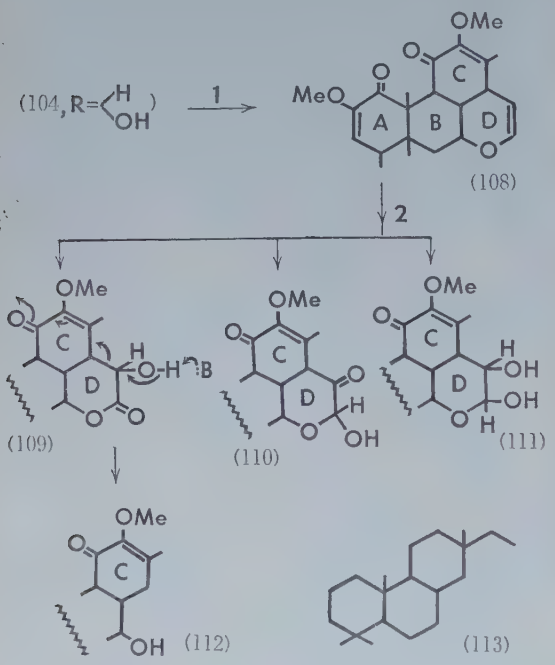

(107)

formulated[51a] from spectroscopic evidence as an enol-lactone (106). This indicates a 1,5 relationship between the carboxyl group and the carbonyl group of the diosphenol group in norquassinic acid (105). A γ-lactone whose infra-red and NMR spectra are consistent with structure (107), is obtained by oxidation of norquassinic acid (105) with lead tetra-acetate. Hence a hydrogen atom is located at C(9).

Oxidation of anhydroneoquassin (108) with potassium permanganate yields three products (109), (110) and (111). Periodic acid oxidation of (111) followed by hydrolysis and then oxidation affords a γ-lactone thus confirming the presence of a δ-lactone in quassin. The relative position of the lactone was revealed by treating hydroxyquassin (109) with a base.

Two carbon atoms were eliminated and a non-lactonic compound (112) was formed, presumably by the mechanism indicated. This evidence, plus the fact that the dehydrogenation of quassin and its derivatives[51] yields 3,4,5-trimethyl-guaiacol and 1,2,8-trimethylphenanthrene, led to structure (104, R = O) for quassin[51a].

It has been suggested that quassin is derived biogenetically from a pimarane skeleton (113) by two 1,2 shifts[51a] .



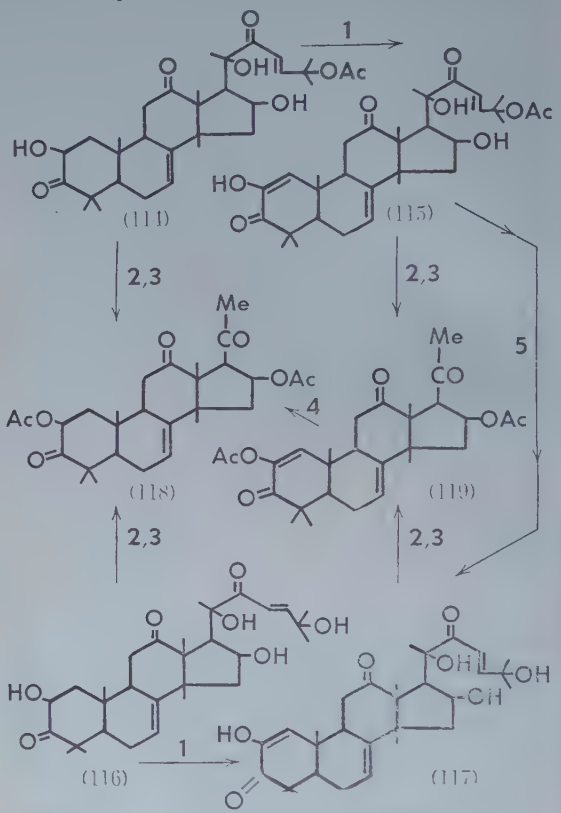
Reagents 1, Ac_2O + $NaOAc$; 2, $KMnO_4$.

5 TRITERPENOID BITTER SUBSTANCES

5.0.1 *The cucurbitacins*

The cucurbitacins are a group of related bitter compounds which have been isolated from various Cucurbitaceae. The group comprises cucurbitacins A, B (114), and C, cucurbitacin D (elatericin A) (116), cucurbitacin E (α-elaterin) (115) and cucurbitacin I (117). Some of these substances have been shown to have anti-tumor activity[1] and elaterin has been used medicinally as a drastic cathartic. The inter-relationships of some of the cucurbitacins have been demonstrated[52] and are summarized in Figure 1.

These compounds have been assumed to be tetra-cyclic triterpenes because 1,2,8-trimethylphenanthrene is formed during selenium dehydrogenation experiments[53]. However, conversion to a known triterpene or a derivative has not yet been achieved. The side chain



Reagents 1, Bi_2O_3; 2, Ac_2O; 3, HIO_4; 4, H_2/Pd; 5, *enzyme.*

Figure 1 *Relations between some cucurbitacins.*

shown in (120) was confirmed by ozonolysis and periodic acid oxidation experiments[54, 55, 56, 57]. This formulation is consistent with ultra-violet absorption spectra and the formation of acetoin when the cucurbitacins are treated with hot alkali[58].

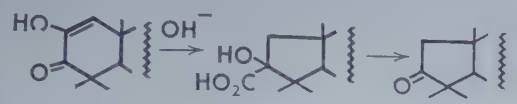
$$Me_2C(OR)CH{=}CHCOC(OH){-}\underset{\displaystyle Me}{\underset{|}{}}$$

(120)

R = Ac for cucurbitacins B and E, R = H for cucurbitacins D and I.

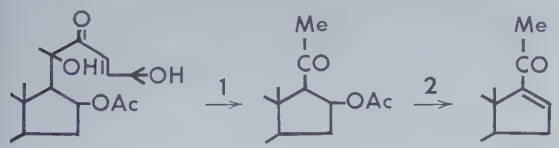
Cucurbitacins E and I have ultra-violet absorption spectra typical of diosphenols in contrast to cucurbitacins B and D. The conversion of cucurbitacins B and D into cucurbitacins E and I, respectively, by oxidation with bismuth oxide (a reagent specific for the oxi-

dation of α-ketols to α-diketones)[59] demonstrated the inter-relationship of these compounds[60, 52]. When treated with hot alkali cucurbitacin E affords ecballic acid[61], a transformation which is explained by the benzilic acid type rearrangement of a diosphenol[62]. This was confirmed by converting ecballic acid into a cyclopentanone derivative (121) as shown. The ultra-violet absorption characteristics of the cucurbitacins are compatible with the placing of the α-ketol and α-diketone groups at C(2) and C(3) in ring A and not in rings B or C[62].



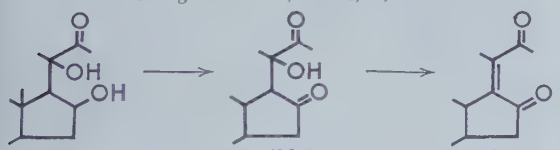
(121)

The presence of a hydroxyl group at C(16) in the cucurbitacins was demonstrated by the formation of an α,β-unsaturated ketone (122) when a periodic acid fission product was treated with acid[52]. Further evidence was provided by oxidizing methyl ecballate (123) to a compound possessing a cyclopentanone system (124). Treatment with alkali converts this compound into an ene-dione (125)[53].



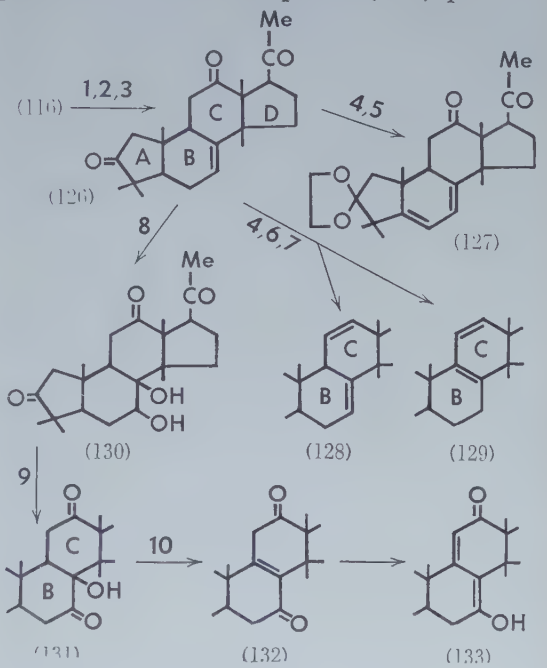
(122)

Reagents 1, HIO_4; 2, H^+



(123) (124) (125)

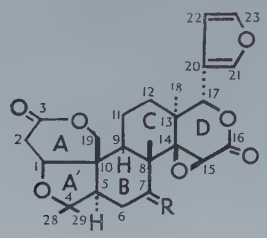
The cucurbitacins possess an unreactive isolated carbonyl group[63] and an inert double bond[56]. Because of its inertness the carbonyl group was assigned to either C(11) or C(12) in the tetracyclic skeleton. Initially C(11) was favoured because the compound (122) exhibited ultra-violet absorption at 240 mμ whereas steroidal Δ^{16}-12-ketones have an abnormally low maximum at 230 mμ[60]. However, subsequent chemical data indicated that the ketone group is at C(12) and the double bond is between C(7) and C(8)[64]. Thus treatment of the 3,20 bis-ketal of the compound (126) with N-bromo-succinimide affords a diene whose ultra-violet and infrared spectra are consistent with the structure (127). When the bis-ketal (126) was converted by lithium aluminium hydride into the corresponding alcohol which was then dehydrated, two dienes were obtained which were formulated with structures (128) and (129) from spectral evidence. The relative positions of the double bond and the ketone group were more conclusively demonstrated by oxidizing the glycol (130) to the hydroxytetraketone (131) which, on dehydration, furnishes the compound (133). The compound (133) presumably arises from the enolization and isomerization of the intermediate compound (132).



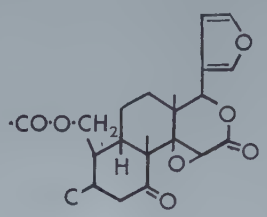
5.0.2 *Limonin*

The citrus bitter principle limonin (134, R=O) was first isolated in 1841[65] and although its chemistry has been investigated intensively during the last ten years[66], its structure has only just been elucidated[67, 68]. The complexity of this compound has required the combined efforts of three groups of investigators. These were led by Professor D. H. R. Barton of London, Professor O. Jeger and Dr. D. Arigoni of Zurich, and Dr. E. J. Corey of Harvard. Simultaneously, Professor J. M. Robertson and his group at Glasgow have

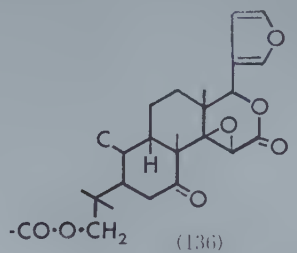
been investigating the structure by X-ray crystallography and they have conclusively elucidated its constitution[68]. At the time of the X-ray team's announcement of the structure the chemical evidence had conclusively proved the presence of the system (135) or (136) in



(134)



(135)



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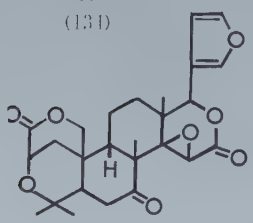

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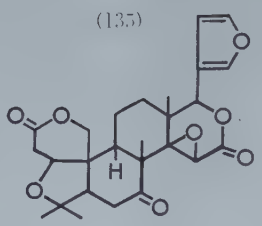

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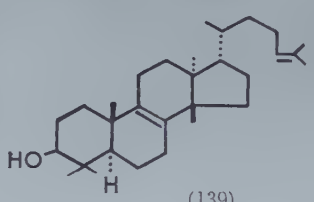


(139)

Reagents 1, HIO_4; 2, OH^-; 3, H_2Pd/C; 4, $HO(CH_2)_2OH/pMeC_6H_4SO_3H$; 5, *N-bromosuccinimide;* 6, $LiAlH_4$; 7, $POCl_3/Pyr$; 8, OsO_4; 9, CrO_3; 10, $pMeC_6H_4SO_3H$

limonin. Based on the hypothesis that limonin is biogenetically derived from a steroid, two structures (137) and (138) were regarded as strong possibilities though chemical evidence did not completely exclude other structures. Nothing was known about the stereochemistry of limonin from the chemical evidence but this of course was revealed by the X-ray studies[68].

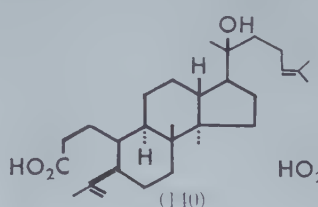
5.0.3 *The biogenesis of limonin*

Limonin, $C_{26}H_{30}O_8$, might at first glance be considered to be a diterpenoid plus an additional six carbon atoms. However, theoretical considerations of its possible biogenesis reveal it to be a degraded triterpene of the euphol (139) type. The scheme envisaged is as follows[67]:

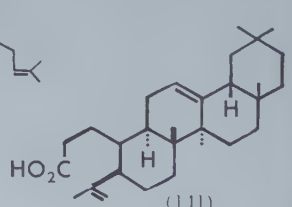
(a) The four carbon atoms at the end of the side chain are removed and the remaining four are converted into a furan ring.

(b) Ring A is modified by oxidative cleavage between C(3) and C(4) and the carboxyl group so formed is oxidatively cyclized to C(19). The feasibility of such a transformation is supported by the occurrence of dammarenolic acid (140) and nyctanthic acid (141) in which cleavage between C(3) and C(4) has taken place.

(c) The formation of Ring D of limonin is envisaged as occurring by the epoxidation of a ring D α,β-unsaturated ketone (142) to give an oxido-ketone (143) which, on undergoing a Baeyer-Villiger type oxidation would be converted into an α,β-epoxylactone (144). Support for this hypothesis is found in the



(140)



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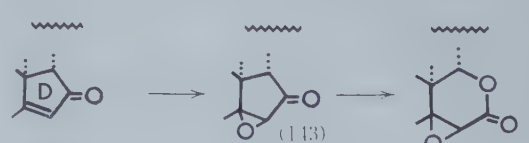
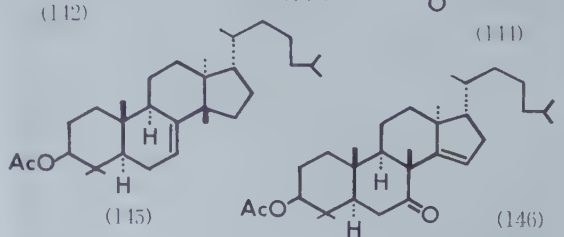


(142) (143) (144)



(145) (146)

oxidation of dihydrobutyrospermol acetate (145) to the 7-ketone (146). In this trans-

formation the necessary methyl migration takes place and the compound (146) could well be oxidized to the α,β-unsaturated ketone (142).

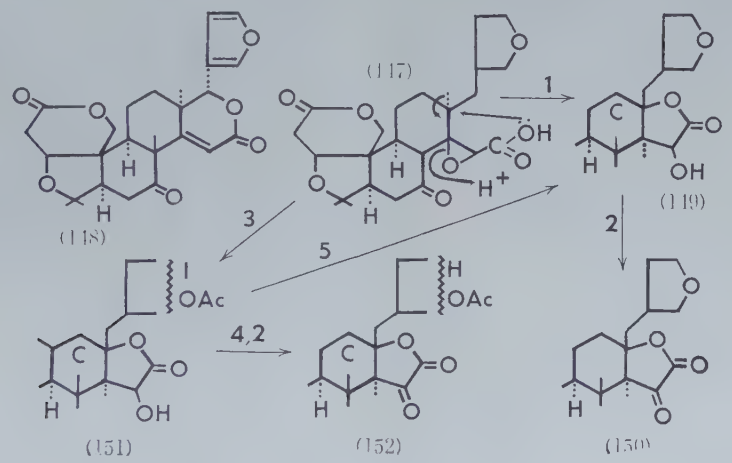
These postulates[67] are consistent with the relative configuration of limonin as revealed by the X-ray studies[68] and the absolute configuration determined by molecular rotatory dispersion studies[67]. Numbering of limonin follows from ideas of its biogenesis and is the same as other tetracyclic triterpenes.

5.0.4 *Chemical evidence for the structure of limonin*

As one might expect from such a structure, limonin undergoes many subtle reactions of unusual interest. The presence of a furan ring, first proposed by Fujita and Hirose[69], was confirmed by ultra-violet and infra-red evidence, by the formation of a diethyl acetylene dicarboxylate adduct which, when pyrolysed furnished 3,4-furandicarboxylic acid[70], and by the production of furan-3-aldehyde when limonol (134, R=(–H,–OH)) was treated with alkali. Furthermore, the NMR spectrum indicates that limonin is a mono-β-substituted furan[71].

From infra-red data the two lactone rings of limonin were shown to be δ-constituted. Since hydrogenation of limonin gives tetrahydrolimonin and hexahydrolimoninic acid (147), in which hydrogenolysis of one of the lactone rings has occurred, the ether oxygen of the cleaved lactone must be in an allylic position to the furan ring[66, 67]. This is analogous to the case of columbin (96).

Because hexahydrolimoninic acid (147) is an unusually strong acid it was deduced that an oxygen substituent was α- to the carboxyl group[66]. This substituent was shown to be an epoxide grouping by treating limonin (134, R=O) with hydriodic acid and obtaining an α,β-unsaturated δ-lactone, desoxylimonin (148)[72]. The formation of desoxylimonin by the reduction of limonin with chromous chloride further substantiated the presence of an epoxide group adjacent to a lactone carbonyl[67]. In keeping with this evidence hexahydrolimoninic acid (147) behaves as a glycidic acid when pyrolysed and affords decarboxyhexahydrolimoninic acid whose NMR spectrum shows it to be an aldehyde. Other chemical evidence confirmed these conclusions.

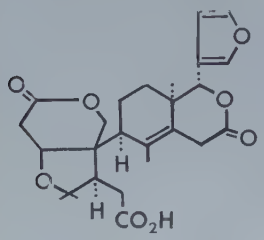


Reagents 1, *HCl-HOAc;* 2, *CrO_3/Pyr;* 3, *HI-HOAc-Ac_2O;* 4, *Zn;* 5, *OH–*

The presence of a methyl group at C(13) was deduced from the results of acid-catalysed rearrangements[73]. Thus, treatment of hexahydrolimoninic acid (147) with hydrochloric–acetic acid yields a neutral isomer (149) whose infra-red spectrum indicates that it contains a γ-lactone. A non-enolizable α-keto-lactone (150) is obtained by the oxidation of (149) and this suggests that a methyl group has migrated to the β-position in the lactone ring. When hexahydrolimoninic acid (147) is treated with hydriodic acid in acetic acid and acetic anhydride, a similar transformation occurrs and the tetrahydrofuran ring opens to give an iodo-acetate (151). This compound

was converted into a non-enolizable α-keto-lactone (152) and the compound (149) as shown.

The location of the carbonyl group was deduced from the fact that mild alkali converts desoxylimonin (148) into desoxylimonic acid (153) which is no longer an α,β-unsaturated δ-lactone[67, 73]. Since desoxylimonin oxime



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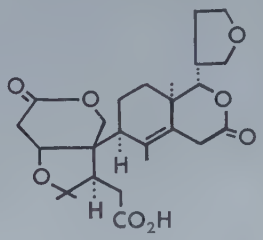

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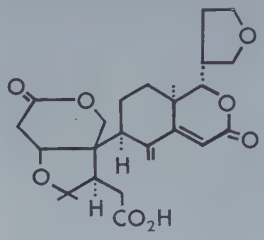
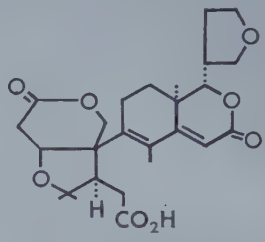


(155)

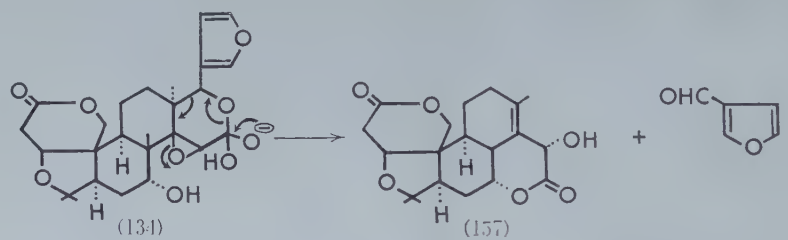


(156)

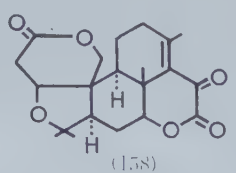
is stable under these conditions the relationship between the ketone group and the α,β-unsaturated lactone in desoxylimonin is revealed. An alternative position for the ketone group is at C(12) but this was rejected on the following evidence[73]. Two diene acids are obtained by heating the adduct formed by the action of chlorine on desoxytetrahydrolimonic acid (154). The ultra-violet spectra of these acids suggest the structures (155) and (156) and exclude the corresponding structures based on a C(12) ketone. Since the *cisoid* diene (155) gives formaldehyde on ozonolysis, the presence of a methyl group at C(8) is proved and the formation of the *transoid* diene (156) demonstrates the presence of a hydrogen at C(9).

A more spectacular demonstration of the relationship between the furan ring, the glycidic lactone and the keto group of limonin results from the action of alkali on limonol (157)[66]. This very interesting reaction is presumably initiated by the attack of a hydroxyl ion on the lactone carbonyl and results in the elimination of furan-3-aldehyde. Ozonolysis of merolimonol (157) gives a methyl ketone thus proving the presence of a methyl group at C(13).

Merolimonol (157) undergoes an interesting reaction with manganese dioxide and gives, besides the expected ketone (158), a decarbonylated derivative (159)[67]. This derivative (159) is converted to a non-enolizable α-keto-γ-lactone (160) by ozonolysis indicating the presence of a carbon substituent at C(8). When treated with barium hydroxide merolimonol (157) gives an hydroxy acid which furnishes a methyl ester (161). Oxidation of this ester with manganese dioxide again results in the unusual decarbonylation reaction to yield the γ-lactone (162) and this can be oxidized to an aldehyde (163). The formation of this aldehyde indicates that C(19) or an equivalent methyl carbon is involved in the ring A lactone.



(134) (157)



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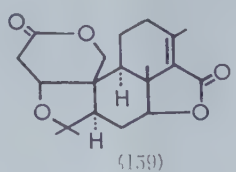

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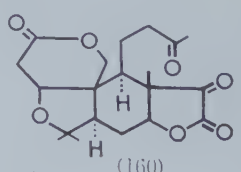
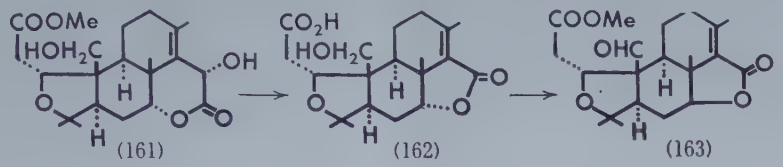


(160)

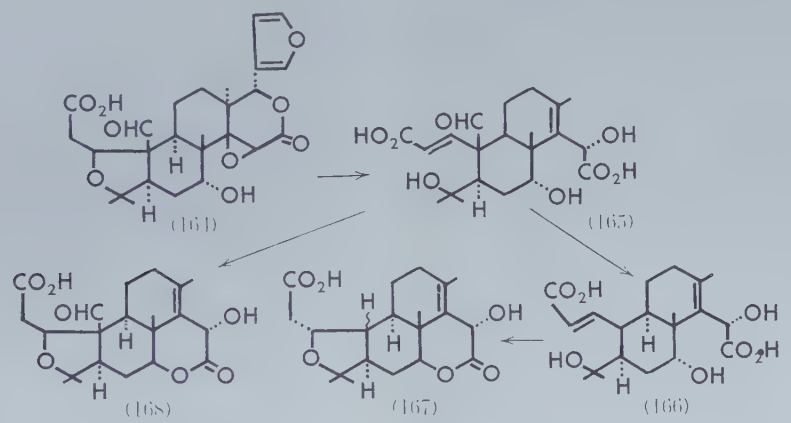
Vigorous treatment with alkali converts limonin (134, R = O) into limoclastic acid (134, (R = O) by oxidation with alkaline permanganate or alkaline hypoiodite[74]. Since



(161) (162) (163)

(167), $C_{20}H_{28}O_6$[66]. Since the chemical behaviour of limoclastic acid is very like that of merolimonol (157) these two compounds were similarly formulated. The loss of the extra carbon atom in the limonin → limoclastic acid transformation is presumed to occur through the deformylation of a compound (165). Such an intermediate could arise

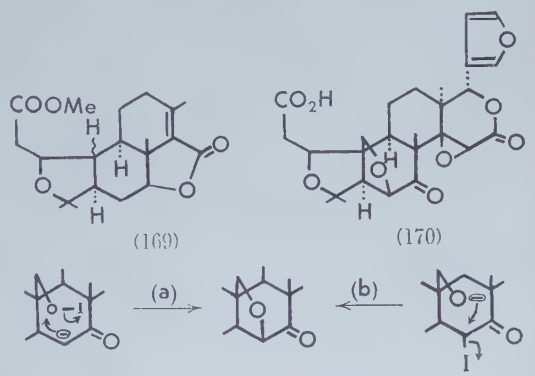
limonilic acid (170) has no hydroxyl absorption in the infra-red region and the absorption maximum of the ketone group is displaced from its normal position at 1710 to 1722 cm^{-1}, the ether ring as in (170) was proposed[67]. In agreement with this, reduction of limonilic acid (170) with aluminium amalgam yielded limonin (134, R = O). Two possible mechan-



from an intra-molecular hydride transfer to the ketone group of limonin to form a compound (164) which then undergoes the limonol → merolimonol change. In support of this hypothesis the aldehyde (168) has been isolated as a minor product of the reaction. The relationship between the limoclastic acid and merolimonol series was demonstrated when treatment of the aldehyde (163) with barium hydroxide followed by methylation of the product yielded the lactone ester (169) obtained by the oxidation of (167) with manganese dioxide. Interpretation of the above phenomena suggests that ring A of limonin must be a δ-lactone with at least one α-hydrogen to permit reversible elimination of a β-ethereal oxygen.

Another fascinating reaction is the formation of limonilic acid (170) from limonin

isms, (a) and (b), have been proposed for the limonin → limonilic acid transformation as shown below (169) and (170).

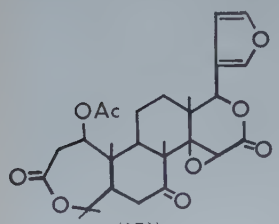


5.0.5 *Nomilin and obacunone*

Nomilin and obacunone are minor bitter principles of citrus species[75]. On the basis of

chemical evidence and the assumption that they are biogenetically related to limonin, structures (171) and (172) have been proposed for nomilin and obacunone, respectively[67, 73]. Whilst these structures have not been proved conclusively they are in full accord with the available experimental evidence[73, 76, 77].

When nomilin (171) is treated with a suitable hot tertiary amine acetic acid is eliminated and obacunone (172) is formed. Both



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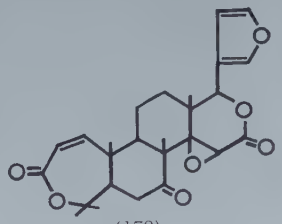
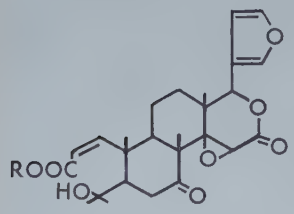
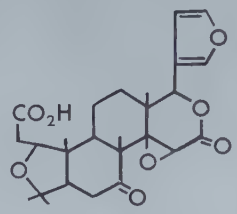


(172)



(173)



(174)

nomilin and obacunone when treated with mild alkali yield obacunoic acid (173, R = H) whose chemical behaviour is very similar to that of limonin[73]. Confirmation of the presence of the seven-membered α,β-unsaturated lactone ring in obacunone (172) comes from the formation of iso-obacunoic acid (174) when methyl obacunoate (173, R = Me) is treated with sodium methoxide. The formation of such an iso-acid was postulated in the biogenesis of limonin.

An interesting feature of obacunone (172) is that it gives a positive iodoform test[76]. Investigations have shown that it is the hydroxyisopropyl group of obacunoic acid (173, R = H) separated by two carbon atoms from the ketone group which is responsible for this reaction[73]. Indeed this reaction appears to be quite general.

6 ADDENDA

6.0.1 *Gentiopicrin*

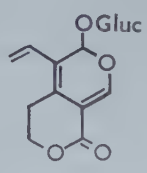
Canonica and his co-workers[78] have proposed a new structure (175) for gentiopicrin (see section 2.0.1) which is compatible with all of the chemical and physical evidence.

6.0.2 *Loganin*

The formula (6) previously proposed for loganin[5] (see section 2.0.2) has now been shown to be incorrect[79] but no alternative structure has been suggested.

6.0.3 *Clerodin*

The constitution and stereochemistry of clerodin, the bitter principle of *Clerodendron infortunatum*, has been established as (176) by X-ray crystallographic investigations[80]. Chemical and nuclear magnetic resonance results are in agreement with this structure[81]. Clerodin is a diterpenoid bitter principle whose biogenesis follows that proposed for columbin.



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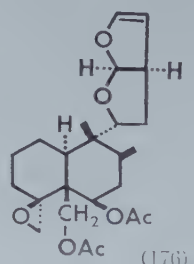

(176)

7 ACKNOWLEDGEMENT

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The Development of Radiation Chemistry in the U.S.S.R.

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1 INTRODUCTION
2 RADIATION CHEMISTRY OF WATER AND AQUEOUS SOLUTIONS
3 RADIATION CHEMISTRY OF SIMPLE INORGANIC MOLECULES
4 RADIATION CHEMISTRY OF ORGANIC COMPOUNDS
5 RADIATION POLYMERIZATION
6 EFFECTS OF IONIZING RADIATIONS ON POLYMERS
7 RADIATION ELEMENTARY ACTS AND PRIMARY CHEMICAL PROCESSES
8 REFERENCES

1 INTRODUCTION

The study of chemical changes brought about by high-energy radiation began to develop rapidly when powerful sources of ionizing radiation became readily available. The development of this new branch of chemistry was stimulated initially by the requirements of nuclear engineering and the need for reliable protection against radiation hazards. Radiation chemistry deals with those phenomena which result from the interaction of highly excited and therefore highly reactive particles, i.e., ions, free radicals and molecules, the excitation resulting from the fact that the energy of ionizing radiation is many times greater than the chemical-bond energy. The generation of ions and possibly excited ions is the main feature which distinguishes radiation-chemical from photochemical processes where such ionization rarely occurs. This circumstance has also contributed to the growth of interest in radiation chemistry.

Russian and Soviet scientists have made a substantial contribution to the development of this field and Orlov was among the first investigators to observe the chemical effects of ionizing radiation. In 1904-1906 he found[1] that a paraffin wax, stearic acid and other solid organic substances were converted into liquid resinous products. In 1910, Zelinsky[2] sealed in a tube some small crystals of radium bromide which contained 0·5 mg of pure radium and 1·5 ml of cyclohexene. Several years later free carbon was found deposited on the surface of the bromide crystals and the mother liquor contained not only hydrocarbons of molecular weight lower than cyclohexene but also heavier hydrocarbons synthesized by the action of α-particles emitted by radium. Dehydrogenation of the original material apparently occurred at an intermediate stage. Zelinsky also pointed out that similar processes could take place in nature due to radiation from radium contained in rocks.

Modern development of radiation chemistry in the U.S.S.R. began in the post-war years, following the development of nuclear power for various purposes. This period has been characterized by a rapid development of research on a wide scale. On the one hand, more diversified classes of compound and of reaction type have been studied; on the other hand, modern research techniques such as gas-liquid chromatography, electron paramagnetic resonance, mass spectrometry have been used for the identification of radiolysis products and for the elucidation of reaction mechanisms. Nuclear reactors, spent heat releasing elements of atomic power reactors, γ-ray sources from ^{60}Co and ^{137}Cs, α-emitters such as radon and polonium preparations, betatrons, electron accelerators permitting both continuous and pulsed irradiation, X-ray installations, have all been employed as sources for ionizing radiation.

Radiation-chemical research laboratories are located in various regions of the U.S.S.R. and several of the Moscow research institutes are working in this field. The Ukrainian S.S.R., Georgian S.S.R., Uzbeck S.S.R. and other republics with atomic reactors are also active and the fact that in the last three years about three hundred articles on this topic have been published by investigators reflects the interest in the subject in this country. In 1957, the first All-Union conference on radiation chemistry was held in Moscow, at which fifty-six papers were reported and discussed[3]. Many of the Soviet investigations in this field have been reported at other scientific conferences both in this country and abroad[4–10].

This paper will, in the main, describe the more important directions of research in radiation chemistry in the U.S.S.R. and also will review some interesting papers published in 1958-1960. Previous Soviet work will be found either in the form of separate collections[11, 12] and transactions of a number of conferences[4–7], or described in reviews[13–16] and booklets[17, 18] published in the U.S.S.R.

2 RADIATION CHEMISTRY OF WATER AND AQUEOUS SOLUTIONS

Many investigations of the radiation-chemical reactions occurring in water and aqueous solutions have been carried out recently in this country. These studies are important since water is used as a moderator and a coolant in atomic reactors; in addition, many processes involved in nuclear fuel production and isolation of nuclear reaction products occur in aqueous solutions. Moreover, the behaviour of aqueous solutions under irradiation is analogous, to a considerable extent, to that of biological systems and can therefore be used as a basis for estimating the effects of radiation on living tissue.

Soviet research work has covered a wide range of problems on the radiation chemistry of water and aqueous solutions: the influence of ionization density and dose rate on radiolytic yields in aqueous solution, the nature of the direct action of radiation on dissolved substances, the role of excited water molecules in radiolytic processes, the dependence of the yields of molecular products of radiolysis on the concentration of the solution. Radiation-electrochemical processes, the influence of irradiation on the corrosion of metals and some other topics have also been investigated.

At present it is generally accepted that the action of radiation on water generates H atoms and OH radicals as well as molecular hydrogen and hydrogen peroxide as primary products of the radiolysis. These intermediate products interact further with compounds in solution. For dilute solutions, it is possible in this way to explain the character and yield of the radiolysis products and, in some cases, the radiolysis kinetics also. For example, Filinovsky and Chizmadzhev[19] calculated the dependence of the yield of molecular products, on this basis, for the radiolysis of water by radiation of high intensity in the presence of scavengers. Two cases of H atom and OH radical distribution during radiolysis were considered: (1) when both radicals lie in a cylindrical track of diameter $\approx$ 1 Å and (2) when the H atoms have a more diffusive distribution ($\approx$ 100 Å).

Ershler *et al.*[20] compared the rate of radiation-chemical decomposition of hydrogen peroxide with the most reliable data available in the literature for the radiolysis of water under the action of γ-radiation from ^{60}Co. They confirmed the applicability of the hypothesis concerning the constancy of yields of the radiolysis products of water, as well as the adequacy of applying laws of homogeneous kinetics to radiation-chemical processes in dilute aqueous solution.

In another paper, based on the model in which free radicals and molecular products are distributed homogeneously in the total irradiated volume, Ershler[21] considered the general regularities of the radiolysis for those cases in which this model is applicable. Proceeding from the assumption that the yields of radiolysis products from water are constant and that in the solution only bimolecular reactions occur, the author considered the effects of radiation intensity on the variation of yield with concentration. Two criteria for the adequacy of this model were found. In the stationary state, an increase of intensity from I_1 to I_2 displaces the curve (logarithm concentration of one product *versus* logarithm concentration of another product) along the coordinate axes by a distance equal to $\Delta I^{\frac{1}{2}}$, the shape of the curve being unchanged. If the stationary state has

not been reached, the variation of intensity leads to displacement by the same value $\Delta I^{\frac{1}{2}}$ along the abscissa of the curve (logarithm radiation yield (G) of one product *versus* logarithm concentration of another). The radiolysis of solutions of hydrogen peroxide[20] provides an example of such a regularity.

It is known that an increase in concentration can bring about conditions in which the dissolved compound will interact not only with free radicals distributed throughout the whole volume of the solution but also with those in the regions of high ionization density, and will thus compete with the recombination reaction of the radicals and reduce the yield of molecular products. In this connection the investigations of Dolin, Bakh, Proskurnin and their collaborators are of considerable interest. For nitrate systems[22–24], as well as for aqueous solutions of potassium bromide[25, 26], it was found that a substantial part of the molecular hydrogen originates from the recombination of H atoms. For example, it was found that $G(H_2)$ decreases to approximately 0·01 molecule/100eV with increasing NO_3 ion concentration[22–24].

Shubin and Dolin[27] elucidated the mechanism of the radiolytic oxidation of Fe^{2+} ions in dilute aqueous solution by investigating the effect of a pressure of hydrogen above the solution on the yield $G(Fe^{3+})$. They found that variation of the hydrogen pressure from 1 to 180 atm does not exert an appreciable influence on the system. A mechanism based on the following reactions was proposed:

$$Fe^{2+} + \cdot OH \rightarrow Fe^{3+} + OH^-$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$$
$$H_2 + \cdot OH \rightarrow H_2O + \cdot H$$
$$H\cdot + H^+ \rightarrow H_2^+$$
$$Fe^{2+} + H_2^+ \rightarrow Fe^{3+} + H_2$$

The results obtained confirm the hypothesis that in deaerated solutions H atoms take part in the oxidation of Fe^{2+} ions either directly or *via* the formation of H_2^+ ions.

It is known that the yields of radical and molecular products of water radiolysis are dependent on the magnitude of the linear energy transfer, i.e., on the type of radiation. Although the number of Soviet investigations on this topic is not large, they are of interest. Kabakchi *et al.*[28, 29] investigated the effects of γ-radiation from ^{60}Co, β-rays from ^{32}P and α-particles from ^{239}Pu on aqueous solutions of nitrates at various concentrations. The radiation-chemical yield of NO_2^- is dependent on the nature of the radiation. For example, α-radiation from ^{239}Pu gives $G(NO_2^-)$ values which are considerably smaller than those from ^{60}Co γ-rays or ^{32}P β-particles.

Ershova and Vladimirova[30] studied the action of α-particles from polonium on 0·8N sulphuric acid and found that the initial yield of hydrogen peroxide (1·2 molecule/100eV) is independent of the concentration of dissolved polonium in the range of specific activity from 0·1 to 12 millicurie/ml, the concentration of hydrogen peroxide tending to a limiting value. The equilibrium limiting value of the H_2O_2 concentration amounts to 5 to 8 $\times$ 10^{18} molecule/ml.

Brusentseva and Dolin[31] investigated the action of 660 MeV protons on aqueous potassium bromide solutions, the linear energy transfer of the radiation being nearly equal to that of 1 MeV electrons. The work demonstrated that these radiation-chemical phenomena are determined solely by the magnitude of the linear energy transfer. Thus the yields $G(Fe^{3+})$ obtained for ferrous sulphate solution and $G(H_2)$ obtained for potassium bromide solutions of various concentration (Figure 1) were found by these authors to be approximately equal to the corresponding values obtained under action of the γ-radiation from ^{60}Co.

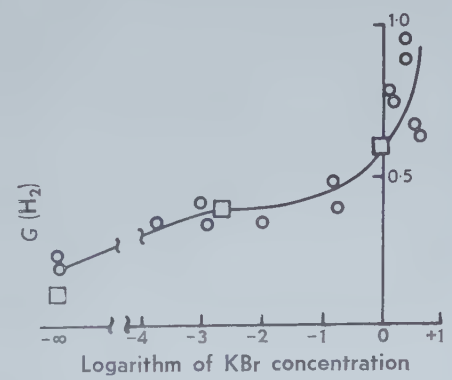


Figure 1 The dependence of $G(H_2)$ on molar concentration of KBr solution under the action of protons with energy 660 Mev (1) and γ-radiation of ^{60}Co (2)

Pikaev and Glazunov[32–37] studied radiolytic transformations of several inorganic compounds in water at high dose rates. These

were achieved by means of pulsed electron radiation (pulsed electron beam, energy 0·8 to 1·8 MeV, duration 5 sec). Radiolytic conversions in aqueous sulphate solutions of Fe^{2+} and Ce^{4+}, of the mixtures Ce^{4+} and Tl^{+} and also Ce^{4+} and Ce^{3+} were also studied. Figure 2 indicates the observed dose rate dependence of $G(Fe^{3+})$, $G(Ce^{3+})$ in the absence of Tl^{+} and

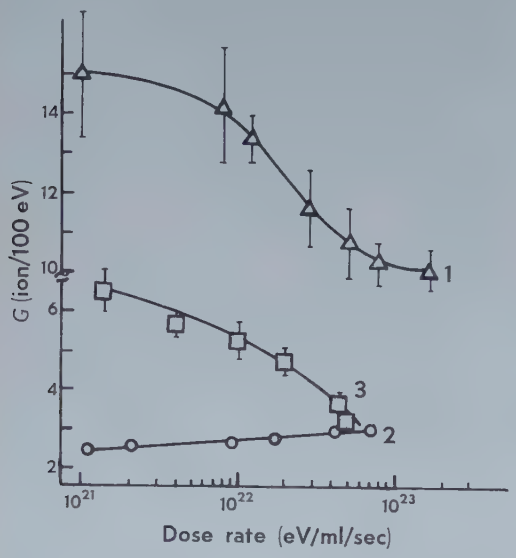


Figure 2 The effect of high dose rates of radiation upon the yields of radiolytic transformations in aqueous 0·8 N sulphuric acid solutions: (1) the dependence of $G(Fe^{3+})$ on dose rate in 3×10^{-3} M solution of ferrous ammonium sulphate; (2) the dependence of $G(Ce^{3+})$ on dose rate in 2×10^{-4} M solution of ceric sulphate; (3) the dependence of $G(Ce^{3+})$ on dose rate in solution of the mixture of $Ce(SO_4)_2$ and Tl_2SO_4 (concentration is 2×10^{-4} and 1×10^{-2} M respectively).

also of $G(Ce^{3+})$ in the presence of Tl^{+}. It can be seen from Figure 2 that appreciable variation of yields occurs at high dose rates above about 10^{21} eV ml^{-1} sec^{-1}. For instance, $G(Fe^{3+})$ decreases with increase of the dose rate, whereas $G(Ce^{3+})$ increases under the same conditions. This is caused by the fact that at the dose rates above about 10^{21} eV ml^{-1} sec^{-1} those processes which originate by overlapping of ionizing particle tracks begin to play a significant part, changing the yields of radiolysis products of water, $G(H)$, $G(OH)$, $G(H_2)$ and $G(H_2O_2)$.

In the papers mentioned above[32–37] it was also recorded that at high dose rates competition between radical-radical and radical-solute reactions becomes more important. Figure 3 shows the dependence of the yields of radiolytic conversion upon the concentration of dissolved substance at high dose rates of radiation for several systems discussed in the above-mentioned papers.

The mechanism of radiolytic transformations in concentrated solutions is not yet elucidated. In this case, both the effects of radiolysis products on the dissolved substance and the direct effect of radiation on the solute must be considered. Kabakchi was one of the first investigators to have considered this direct action[38]. He showed that in the radiolysis of aqueous chloride solution, molecular chlorine is formed, the yield increasing with solute concentration independently of the type of radiation or of the nature of the cation.

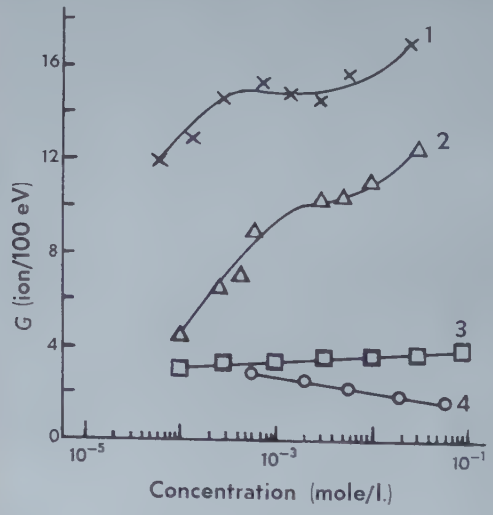


Figure 3 The dependence of the yields of radiolytic transformations on concentration of solution at high dose rates of radiation: (1) the effect of concentration of ferrous ammonium sulphate in aqueous 0·8 N sulphuric acid solution saturated with air on $G(Fe^{3+})$ at dose rate 10^{21} eV ml^{-1} sec^{-1}; (2) the same at dose rate 3.5×10^{22} eV ml^{-1} sec^{-1}; (3) the effect of concentration of thallous sulphate in 0·8 N sulphuric acid solutions of the mixture of Ce^{4+} and Tl^{+} salts saturated with air on $G(Ce^{3+})$ at dose rates 4.5×10^{22} eV ml^{-1} sec^{-1}; (4) the effect of concentration of cerous sulphate in 0·8 N sulphuric acid solution of the mixture of Ce^{4+} and Ce^{3+} salts on $G(Ce^{3+})$ at dose rate about 5×10^{22} eV ml^{-1} sec^{-1}.

A different point of view was suggested by Proskurnin *et al.*[14, 23, 24, 39–43]. According to their hypothesis, at high concentrations it is

necessary to consider not only radicals but also excited molecules, produced by the radiation, to be involved in the radiation-chemical reactions. Figure 4 shows the dependence found[24] between the yield $G(NO_2^-)$ and the concentration of sodium nitrate. The radiation yield

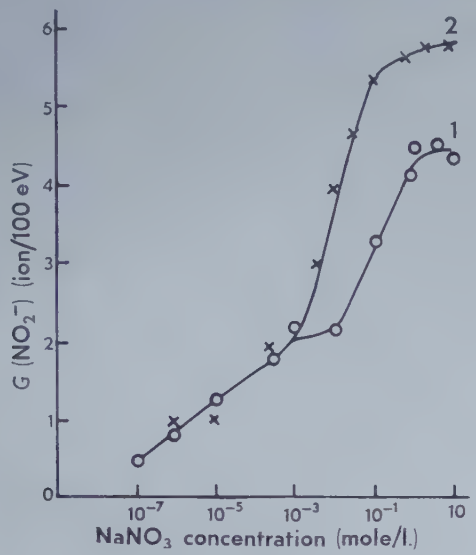


Figure 4 The dependence of $G(NO_2^-)$ on concentration of $NaNO_3$ in solution saturated with nitrogen (pH = 14): (1) in absence of glycerol, (2) in presence of glycerol at 10^{-3} M concentration.

$G(NO_2^-)$ increases with increase in sodium nitrate concentration reaching a constant value in the region 5×10^{-4} to $5 \times 10^{-2}M$. The authors suggest that the existence of this slightly sloping segment of the curve can be explained by combination of practically all the H atoms formed in the reaction with nitrate ions. In the more concentrated solutions the yield $G(NO_2^-)$ increases again and for 1 to 6*M* solutions remains constant. According to these authors[24] the increase in $G(NO_2^-)$ in the concentrated solutions is caused by the H atoms from both ionized and excited molecules of water being involved in the process.

Proskurnin discovered the radiation sensitization phenomena described below. If a substance capable of reacting rapidly with one of the radical radiolysis products of water is added to the solution, conditions are created in which radicals become more completely involved in radiolytic conversion. In the case of nitrate systems[14, 39, 44] such substances are glycerol and glucose. The presence of these active acceptors of OH radicals gives higher $G(NO_2^-)$ values.

Curve 2 in Figure 4 represents the dependence of $G(NO_2^-)$ on concentration of sodium nitrate solution in the presence of glycerol. Here the yield of $G(NO_2^-)$ for highly concentrated solutions reaches a value of about 6 ion/100 eV, which implies that all the water radiolysis products are involved in the reaction with the dissolved substance.

Recently, Proskurnin *et al.*[45] proposed the hypothesis that there are two kinds of excited water molecules differing both in energy and type of excitation. This hypothesis can explain the existence of three limiting values of the radiolysis yield of dissolved substance under various conditions of radiolysis. For the nitrate system above, the limiting values of $G(NO_2^-)$ are equal to 2, 4, and 6 ion/100eV respectively.

Radiolytic transformations in concentrated aqueous solution were investigated by a number of other Soviet scientists. Bugaenko and Belevsky[46] examined the interesting problem of the effects of radiation on concentrated perchloric acid solutions.

It was found by Balandin, Spitsyn *et al.*[47] that irradiation of aqueous solutions of several complex salts of platinum precipitates metallic platinum. Platinum and palladium blacks prepared in this way have higher catalytic activities for low-temperature cyclohexene hydrogenation than when prepared in the conventional way[48].

Radiolytic transformations in aqueous solutions of plutonium were examined by Bakh and his collaborators[49, 50]. Several papers by Soviet authors are devoted to the radiolysis of aqueous solutions of uranium[51, 52]. Firsov and Ershler[51] examined the radiation-chemical reactions in aqueous solutions of uranium (IV) and found that under the action of γ-radiation of ^{60}Co, OH radicals and hydrogen peroxide are responsible for oxidizing U^{4+} ions. As the concentration increases, the oxidation yield at first increases and subsequently decreases. In the presence of oxygen, $G(UO_2^{2+})$ is significantly greater (16 equiv./100eV) than in degassed solution. The authors deduced an equation describing the dependence of $G(UO_2^{2+})$ upon U^{4+} concentration, the ratio of the rate constants of the three reactions ($U^{4+} + \cdot OH$, $H\cdot + \cdot OH$ and $H\cdot + \cdot H$) being involved.

Yakovlev with collaborators[53] examined the effects of α-radiation from ^{241}Am on the valency state of americium in aqueous solution and found that radiation-chemical reduction of AmO_2^{2+} ions depends to a considerable degree on the acidity of the solution as well as on the nature of the acid. For example, in concentrated solutions of perchloric acid, the yield $G(AmO_2^{2+})$ is appreciably lower than that in dilute solution. The reduction of NpO_2^{2+} ions in aqueous solution induced by electron radiation[54] is also dependent on the factors mentioned above. It is of interest that, from a radiation-chemical point of view, neptunium in the pentavalent state is more stable than in its other valency states.

Radiation-chemical processes in aqueous solution have been studied by several Soviet workers. Bakh *et al.*[22, 55] examined the change in oxidation-reduction potential of several compounds in aqueous solution under the action of ionizing radiation. Extensive investigations of radiation-electrochemical processes were carried out by Veselovsky and co-workers[56–61]. They discovered that the potential of a platinum electrode in an irradiated 0·8 *N* sulphuric acid solution saturated with nitrogen reaches a value close to the reversible hydrogen potential, while the potential of a gold electrode becomes equal to 0·95 V.

Kokoulina, Dolin and Frumkin[62] examined the behaviour of smooth platinum electrodes in sulphuric acid solutions over a wide range of dose absorptions and dose rates, and proved that the potential of a platinum electrode in irradiated 0·8 *N* sulphuric acid is controlled by the molecular radiolysis products of water accumulating in solution, i.e. by hydrogen and hydrogen peroxide. The radical products of radiolysis do not play any appreciable part in establishing the potential on platinum in this case. A similar conclusion was reached by Levina and Kalish[63] from an examination of the behaviour of nickel electrodes under irradiation. From a study of the oxidation-reduction reaction $Fe^{2+} \rightleftharpoons Fe^{3+}$ under irradiation, Dolin and Duzhenkov proved that it should be possible to use the oxidation-reduction reaction in aqueous solution to transform radiation energy into electrical energy. In other words, the possibility of constructing a radiation-galvanic cell was demonstrated. Another solution of this problem was advanced by Veselovsky *et al.* who employed platinum and gold electrodes having selective properties with respect to the oxidizing and reducing agents formed in solutions undergoing irradiation.

There are a number of Soviet papers which discuss the influence of ionizing radiation on the corrosion of metals in various media. Tomashov, Byalobzhesky *et al.*[65] studied the effects of electron radiation on the corrosion of iron, stainless steel and aluminium in sodium chloride solutions. Byalobzheshky[66, 67] found that the corrosion of several metals in air is appreciably accelerated by ionizing radiation (fast electron beam, γ-rays from ^{60}Co, X-rays). The metals examined can be arranged in the following sequence of increasing corrosion stability: iron < copper < zinc < aluminium. It was suggested that the intensification of corrosion under irradiation is caused by the formation of the radiolysis products of oxygen, water and nitrogen in the surrounding air.

Rozenfeld and Oshe[68, 69] examined the effect of electron radiation on electrochemical activity in various media for zirconium and titanium covered with oxide films having semi-conducting properties. Diminution of the anode and cathode reaction overvoltages and an increase in the rate of these reactions were found. The effect is reversible; removal of the irradiation leads to overvoltages and rates of anode and cathode reactions which approximate closely to the original ones. Another phenomenon of interest discovered by these workers, is that under irradiation, the behaviour of metals covered with semi-conductor films of *p*-type and of *n*-type, is quite different with respect to the anode process. This difference is not observed in the cathode reaction.

The corrosion behaviour of several metals under irradiation was also studied by Kolotyrkin *et al.*[41, 70]. For example[41], they investigated the effects of γ-radiation of ^{60}Co on the electrochemical properties and the corrosion of steel, nickel and platinum, in sulphuric acid. It was established, in the case of steel and nickel, that the effect of irradiation is equivalent to anode polarization. These workers confirmed the conclusion of Veselovsky and his collaborators[56–61] that in sulphuric acid a platinum electrode potential is established close to the potential of the reversible hydrogen electrode.

3 RADIATION CHEMISTRY OF SIMPLE INORGANIC MOLECULES

A number of Soviet workers studied radiation-chemical reactions of simple inorganic molecules. These investigations attracted attention because it was possible to correlate the yields and kinetics with ionization and excitation potentials, dissociation energy and other parameters of the irradiated material, as well as to study the effect of physical state on radiolysis and to correlate radiation-chemical and photochemical processes with these compounds.

The most complete investigation in this field is the radiation-chemical oxidation of nitrogen by oxygen. Pshezhetsky and Dmitriev[71, 72] examined the kinetics and mechanism of the oxidation of nitrogen under the action of electron impact. The nitrogen pressure was varied from 10^{-2} to 10^{-3} mm Hg, with electron energy from about 1 eV to 200 KeV; N_2^+ ions appear to be intermediates in the reaction. Kinetically, the reaction follows an equation of the second order with an activation energy of 2 to 7 kcal/mole. Later, Dmitriev and Pshezhetsky[73] studied the oxidation of nitrogen induced by γ-radiation of ^{60}Co at pressures up to 150 atm and at temperatures, 15 to 25°C and 150°C. It has been observed that increasing the pressure from 1 to 760 mm Hg lowers the oxidation-reaction yield, but further increases of pressure increase the yield to 5 to 6 molecules of NO_2/100 eV at 150 atm. The yield of N_2O at 3 to 50 atm at 150°C is 2·5 to 3·5 molecule/100eV. The dependence of reaction rate on mixture composition follows an equation of the second order. A departure from second-order kinetics is observed in the dependence of reaction rate and yield upon the pressure, due to recombination of ions (produced by the irradiation).

Recently, Dmitriev and Pshezhetsky[74] examined the reactions of nitrogen dissolved in water under the action of γ-radiation from ^{60}Co and of a beam of fast electrons. It was found that nitrogen is fixed as nitrite, nitrate and ammonia. The yield is dependent on the composition of the dissolved gas and its pressure. For example, the yield of fixed nitrogen at 1atm. is 0·09 to 0·18, and at 150 atm, 1·0 to 1·4 atom/100 eV of radiation energy absorbed.

Pshezhetsky *et al.*[75, 76] also studied the formation of ozone in liquid and gaseous oxygen under the action of γ-radiation from ^{60}Co and of fast electrons. Excited molecules of oxygen were proved to play an important role in the reaction. The radiation-chemical yield of ozone is 12 to 15 molecule/100 eV in the liquid phase and 1·5 molecule/100 eV for the gas.

Hydrazine is formed by the action of fast electrons on liquid ammonia[77]. The latter process is characterized by a stationary hydrazine concentration produced by the equilibrium between formation and decomposition, the yield being 1·0 to 1·2 molecule/100 eV.

The kinetics of the decomposition of aqueous hydrogen peroxide by γ-radiation from ^{60}Co or ultra-violet light, like the analogous thermal decomposition[78], depends upon concentration. As the concentration increases the rate of the reaction passes through a maximum. It was also observed that the rate of the radiation-chemical reaction is proportional to the square root of the radiation intensity. The activation energy of the radiation-chemical reaction is 6·5 kcal/mole, that of the photochemical reaction 8 to 9 kcal/mole. The yield of the radiation-chemical reaction is dependent on temperature. The variation in the degree of electrolytic dissociation to HO_2 radicals with concentration is advanced in this paper as an explanation of the kinetics of hydrogen peroxide decomposition.

Several papers by Soviet authors deal with the effect of ionizing radiation on solid substances. Spitsyn *et al.*[79] examined the decomposition of solid complex compounds of platinum under electron bombardment and the formation of free metallic platinum was sometimes observed. The initial yields of metal are given in Table 1. The data show that the degree of

Table 1 Initial yields of free metallic platinum in the irradiation of complex compounds

Compound	Initial G(Pt) (atom/100eV)
$K_2[PtCl_4]$	0·018
$(NH_4)_2[PtCl_4]$	0·143
$(NH_4)_2[PtCl_6]$	0·090
$[Pt(NH_3)_4]Cl_2$	0·047
cis-$[Pt(NH_3)_2Cl_2]$	0·036
trans-$[Pt(NH_3)_2Cl_2]$	0·338

decomposition of the complexes depends on the nature of the salt and its geometric isomerism. For instance, *trans*-$[Pt(NH_3)_2Cl_2]$ has a lower radiation-chemical stability than the *cis*-isomer.

The data plotted in Figures 5 and 6 reveal that the degree of decomposition of the complex salts becomes constant at high irradiation doses.

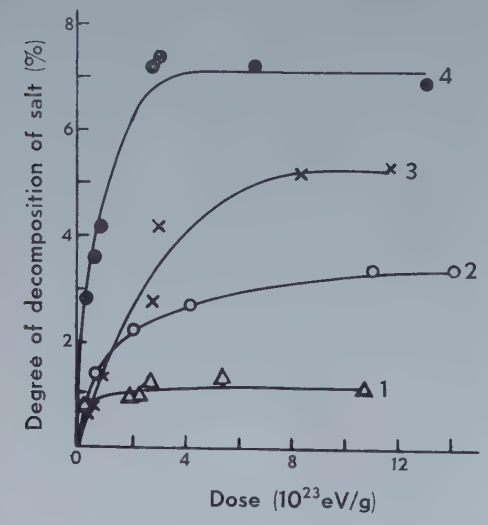


Figure 5 Decomposition of complex salts of platinum under the action of high energy electron beams depending on integral dose at 145 *to* 150°*C.* (1) $(NH_4)_2\,[PtCl_6]$; (2) $[Pt(NH_3)_4]\,Cl_2$; (3) cis-$[Pt(NH_3)_2Cl_2]$; (4) trans-$[Pt(NH_3)_2Cl_2]$.

Under these conditions reoxidation of free metallic platinum by atomic chlorine produced by the radiation-chemical decomposition of the compounds concerned apparently plays a significant part.

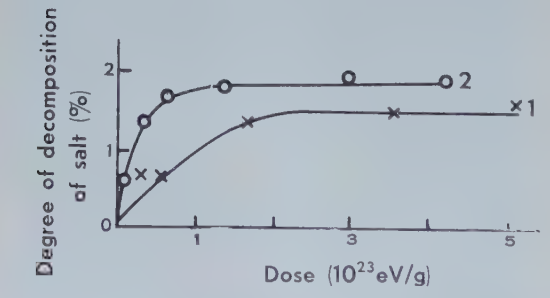


Figure 6 Decomposition of potassium chloroplatinate (1) *and ammonium chloroplatinate* (2) *under the action of high energy electron beams at* 90 *to* 95°*C.*

Proskurnin *et al.* investigated the effect of γ-radiation from ^{60}Co on solid sodium nitrate and potassium chlorate plus potassium perchlorate[80–82], as well as on crystalline hydrates of nitrate salts[83]. The effect of dose rate of γ-radiation from ^{60}Co in the region of $0{\cdot}2 \times 10^{16}$ to $4{\cdot}1 \times 10^{16}$ eV ml^{-1} sec^{-1} on the yields of several crystalline hydrates of nitrate salts is described[83]. It is shown that a minimum in the curve expressing the dependence of $G(NO_2^-)$ upon dose rate is a characteristic feature of all the crystalline hydrates examined, the position of the minimum being determined by the nature of the cation. The crystalline hydrates are more sensitive to the action of γ-radiation than the corresponding anhydrous salts.

Boldyrev *et al.*[84] considered the effect of irradiation pretreatment on the rate of thermal decomposition of silver oxalate contaminated with cadmium. Pretreatment with γ-rays from ^{60}Co brings about a marked increase in the subsequent rate of thermal decomposition of pure silver oxalate. Thermal decomposition of the salt is accelerated by the cadmium impurity alone, but to a much smaller degree. In the opinion of the authors, the phenomenon is caused by the formation, in the presence of cadmium in the crystal lattice, of an additional number of cation vacancies which can trap positive holes or silver cations from interstitial sites.

4 RADIATION CHEMISTRY OF ORGANIC COMPOUNDS

The action of ionizing radiation on organic compounds is being investigated by Topchiev and Poak. They and their collaborators[85–92] are carrying out a systematic investigation of the effects of γ-radiation from ^{60}Co and of fast electrons on hydrocarbons in the gaseous, the liquid and the solid state. They suggest the following types of primary process in the irradiation of normal alkanes.

$$RH \rightsquigarrow RH^* \begin{cases} \rightarrow R\cdot + \cdot H \\ \rightarrow R_n + R_m \\ \rightarrow H_2 + C_nH_{2n} \\ \rightarrow 2H_2 + C_nH_{2n-2} \end{cases}$$

Examination of electron spin resonance spectra (ESR-spectra) of frozen *n*-heptane irradiated at 77°K indicates the presence of free alkyl radicals and also of a smaller number of H atoms. It has been proved that these radicals can be kept at low temperatures for a long time.

These same authors, as well as foreign workers, find that the total yield of the radiolysis products of hydrocarbons at room temperature is about 10 molecule/100 eV, the

predominant process being dehydrogenation. The radiolysis of *n*-heptane provides the most detailed information and under ordinary conditions the gaseous radiolysis products contain about 80% hydrogen, the residue consisting of saturated and unsaturated hydrocarbons.

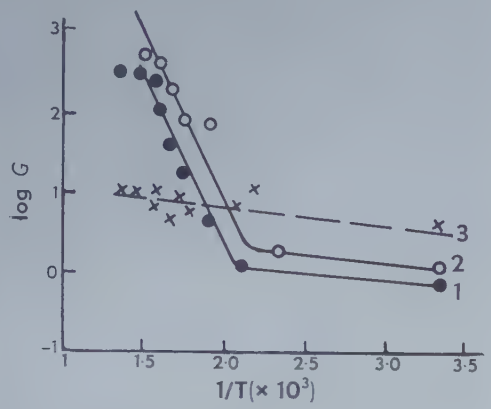


Figure 7 The dependence of the logarithm of radiation-chemical yield of methane (1) *hydrogen* (2) *and hydrocarbon fraction* C_2-C_6 (3), *on inverse temperature of the radiation thermal cracking.*

In the radiation chemistry of hydrocarbons thermal cracking is of significant interest[90–92]. It was found that at elevated temperatures the radiation initiates cracking which continues as a chain reaction maintained by its own exothermicity. For instance, as one can see from Figure 7, the yield of hydrocarbon products from *n*-heptane increases rapidly with increasing temperature. In particular, the proportion of unsaturated hydrocarbons increases with temperature (Figure 8). It might be predicted that the thermal cracking of hydrocarbons under irradiation will have practical applications.

Another branch of radiation chemistry developed by Soviet scientists is the study of radiation-induced oxidation processes in organic systems. Bakh and his co-workers[93–99] studied these oxidations using various types of radiation: X-rays, γ-radiation from ^{60}Co, fast electrons, mixed radiation from a nuclear reactor. They were the first to show[93, 94] that the irradiation of liquid hydrocarbons (heptane, isooctane, benzene, cyclohexane, etc.) saturated with oxygen resulted in the oxidation of the hydrocarbons at room temperature giving per-

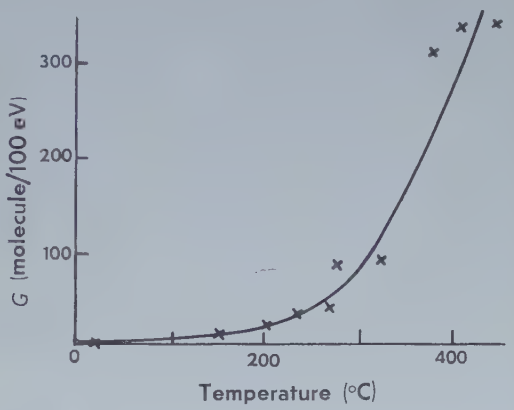


Figure 8 The dependence of radiation-chemical yield of liquid olefins upon temperature of the radiation thermal cracking.

oxides, carbonyl compounds, alcohols, acids, etc., these compounds being formed simultaneously from the beginning of irradiation. Under normal conditions the oxidation of hydrocarbons is not a chain reaction. Ethyl alcohol and acetone, molecules which contain more labile hydrogen atoms, are oxidized by a chain mechanism with short chains. The following scheme suggested by Bakh for radiation-induced oxidation is based on the postulate that peroxide radicals are formed by the addition of oxygen molecules to the free hydrocarbon radicals. The simultaneous formation of the radiolysis products is caused either by parallel reactions of the peroxide radicals of one type or by simultaneous formation of various peroxide radicals.

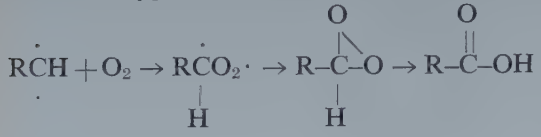
$$R\cdot + O_2 \rightarrow RO_2\cdot$$

$$RO_2\cdot + RH \rightarrow RO_2H + R\cdot$$

$$\begin{array}{l} R''' \\ | \\ R\text{–}CO_2\cdot \\ | \\ R'' \end{array} \rightarrow \begin{array}{l} R' \\ | \\ R'\text{–}CO_2R''' \\ | \\ R'' \end{array} \rightarrow \begin{array}{l} R' \\ | \\ R'\text{–}C{=}O \\ | \\ R'' \end{array} + R'''O\cdot$$

$$RO_2\cdot + \cdot R' \rightarrow RO_2R'$$

The biradical, $R\dot{C}H$, which can also arise during irradiation of hydrocarbons, can interact with oxygen to give a molecule of acid:



Oxidation of methane by oxygen at room temperature under the action of fast electrons was studied by Mikhailov *et al.*[100–102]; no chain reaction was observed.

Bakh and Proskurnin are independently investigating radiation-induced oxidation of organic compounds in aqueous solution. Oxidation is mainly caused by the interaction of the radiolysis products of water with the dissolved substances so that oxidation can take place even in the absence of molecular oxygen.

Proskurnin *et al.*[103, 104] conducted a detailed examination of the radiation-chemical oxidation of benzene to phenol in aqueous solution. They observed an interesting phenomenon: the yield of phenol in the presence of Fe^{2+} ions is three times the normal yield and equals 6 molecule/100 eV. The radiation-induced oxidation of chlorobenzene in aqueous solutions was also examined[105] and it was proved that the hydrolysis of chlorobenzene is an important factor in this radiolysis.

Bakh *et al.*[106] investigated the oxidation of ethyl and isopropyl ether in aqueous solution under the action of X-rays and fast electrons. In the presence of oxygen the oxidation proceeds by a chain mechanism. The products of the reaction in both cases are peroxides (yield about 20 molecule/100 eV), carbonyl compounds (yield about 25 molecule/100 eV), alcohols and acids.

Emanuel[107] and Proskurnin *et al.*[108, 109] showed that at high temperatures chain oxidation of irradiated hydrocarbons takes place. For instance, it was observed that under the action of γ-radiation paraffin in the presence of oxygen is effectively oxidized at 127 to 130°C to fatty acids. The process of oxidation proceeds by a degenerate chain-branching mechanism.

Recently, Bakh and Tung Tyan'-chzhen'[110] examined the temperature dependence of the radiation-chemical oxidation of *n*-heptane and *n*-nonane. For both hydrocarbons there are two regions of temperature dependence of the yield of oxidation products. At temperatures below 70°C small yields independent of temperature are observed. Above 70°C the yield increases sharply with temperature, reaching the value of 30 to 40 molecule/100 eV for peroxides. For the radiation-induced oxidation of *n*-alkanes interference of chain and ordinary reaction mechanisms is a distinctive feature. The temperature at which chains are initiated depends on the irradiated compound.

The work of Bagdasarian and his collaborators[111–113] on the effects of ionizing radiation on mixtures of organic compounds is of considerable interest. From an examination of γ-radiation-induced decomposition of benzoyl peroxides in various solvents they find that in benzene solutions effective transfer of excitation energy from benzene to peroxide molecules occurs, increasing significantly the radiation-chemical yield of its decay. In the reactions studied, excitation energy transfer is observed if molecules of both the constituents of the solution have aromatic groups. For example, in cyclohexane and ethyl acetate solutions, energy transfer from the solvent does not occur and a chain reaction of peroxide decomposition is induced by radicals formed from the solvent. However, a simple correlation between the structure of a molecule and its ability to accept excitation energy does not exist[113]. For example, azobenzene does not accept excitation energy, although its molecules contain phenyl rings conjugated through a double bond. Some compounds, although potential acceptors of the energy, can not (or only to a negligible extent) use the energy for chemical transformation. For instance, phenylazotriphenyl methane does not dissociate into radicals when excitation energy is transferred to it in spite of the fact that dissociation occurs readily when it is heated.

Bagdasarian *et al.*[114] recently described the radiolysis of dilute benzene solutions of organic disulphides containing aromatic and aliphatic substituents. Due to energy transfer from the solvent to the dissolved compound, the yields of disulphide decomposition products are higher than if all the energy absorbed by the disulphide itself had caused decomposition. The energy transfer efficiency is dependent on the nature of the disulphide. Thus, diphenyldisulphide and dibenzyldisulphide have approximately equal

efficiencies while that of dibenzoyldisulphide is only half of this value. Aliphatic disulphides (diethyldisulphide, dioctyldisulphide) show lower decomposition yields than aromatic disulphides due to a considerably smaller intermolecular energy transfer efficiency.

A number of papers have been published dealing with radiation-chemical halogenation, amidation, etc. Zimakov *et al.*[115] examined the effects of γ-radiation from ^{60}Co on the chlorination of benzene at temperatures from —80 to +40°C. Chlorination proceeds with a yield of 4×10^5 molecule/100 eV, the resulting material having a greater content of the highly toxic γ-isomer of hexachlorocyclohexane than the industrial product obtained photochemically.

The effects of ionizing radiation on mixtures of benzene and ammonia, benzene and carbon tetrachloride, and benzene and carbon tetrachloride with compounds containing fluorine, have been examined by Zimin with his collaborators[116–118]. During the γ-irradiation of benzene and ammonia, aniline is formed in small yield. In the case of the benzene-carbon tetrachloride system, formation of monochlorobenzotrichloride (as well as some other radiolysis products) was observed. In mixtures of carbon tetrachloride with inorganic fluorides and of benzene with simple fluorocarbons, fluorination of carbon tetrachloride and of benzene takes place.

Dzhagatspanian *et al.*[119] studied the chlorination of organosilicon monomers and polymers under γ-irradiation. The radiation-chemical sulpochlorination or sulphooxidation of paraffins as well as of some polymers are also described[120].

New radiation-chemical methods of organic synthesis are being sought in this country. Kocheshkov *et al.*[121] proposed a method for the preparation of organotin compounds, and Spitsyn *et al.*[122] worked out a radiation-chemical synthesis of the butyl ether of phosphonitrile chloride. This last reaction proceeds, in good yield, by the action of fast electrons on a solution of phosphonitrile chloride in *n*-butyl alcohol.

Radiation-chemical transformations in dyes and substances of biochemical interests are also being studied. Vereschinsky[123] examined the synthesis and transformation of leuco-compounds of the dyes under the action of γ-radiation from ^{60}Co as well as the effects of γ-radiation on the synthesis of indophenol[124]. In a number of papers[125–128] the radiolysis of methylene blue in aqueous solution is described. Proskurnin *et al.*[129] examined the radiolytic decoloration of indigo carmine solutions. Many investigations have been made of the effects of ionizing radiation on substances of biological interest, e.g., proteins[130], amino acids[131], carbohydrates[132], animal fats[133], etc., and the radiolysis of protoporphyrin has been examined by Vereschinsky[134].

5 RADIATION POLYMERIZATION

Polymerization initiated by radiation, at present, ranks high among the problems to be solved before ionizing radiations can be of industrial use. It has been shown in a number of papers, including some publications by Soviet authors[135], that in the majority of cases radiation-induced polymerization proceeds by a chain mechanism, the process being initiated by free radicals formed by irradiation of the monomer.

Researches in radiation polymerization are being carried out in many countries. In the U.S.S.R. workers in this field are Medvedev[135, 136], Bagdasarian[137, 138], Abkin[139–141], Tsetlin[142] and others.

Among the latest investigations on this topic that of Tsetlin *et al.*[142], concerning radiation-chemical polymerization of methyl methacrylate, is of considerable theoretical and practical interest. In the presence of air, irradiated methyl methacrylate does not polymerize. However, spontaneous polymerization of the irradiated material proceeds at room and lower temperatures to a high degree of conversion when access of air to the material is prevented. Investigations of the kinetics of radiation polymerization at various temperatures indicate that, to a first approximation, the process is identical in character with the more conventional types of free-radical polymerization. Polymerization is initiated by the products of radiation-chemical oxidation of methyl methacrylate (probably peroxides formed by interaction of primary free radicals with oxygen). Being highly active, these products permit low-temperature polymerization to occur. A

feature of interest is that curves representing the polymerization kinetics are in this case smoother than those of polymerization initiated by benzoyl peroxide. The 'gel-effect' in this process only takes place at much higher degrees of conversion when the ratio of the maximum reaction rate in the self-acceleration stage to the initial rate is smaller. The curves representing the dependence of the ratio of the polymerization rate at a given time to the initial rate are shown in Figure 9.

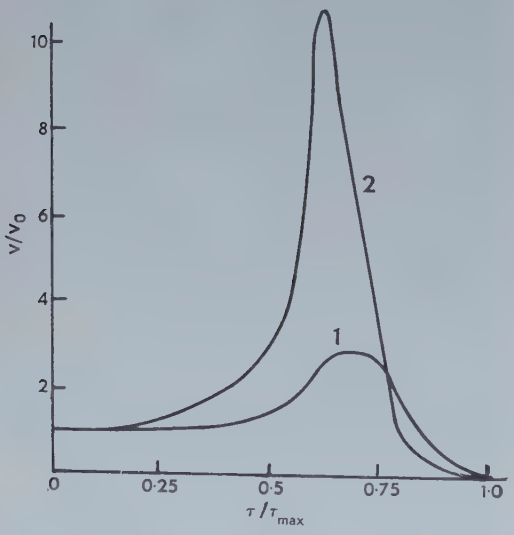


Figure 9 The influence of mode of initiation on the shape of the kinetic curves of polymerization of methyl methacrylate at 60°C: (1) *polymerization of pre-irradiated monomer* (*dose* 4×10^{20} *eV/cm*^{3}); (2) *polymerization of methyl methacrylate in presence of benzoyl peroxide* (0.01%). v/v_0 *is the ratio of the polymerization rate at a given moment to the initial rate;* τ/τ_{max} *is the relative duration of polymerization process.*

Recently, Medvedev *et al.*[143] studied the polymerization of ethylene by γ-radiation from ^{60}Co. In this work the rates of radiation polymerization of ethylene both in solution and in the gas phase at various concentrations and pressures, as well as some properties of the resulting polymers, were examined. At a pressure of 50 atm, a temperature of 25°C and a dose rate of 98 roentgen/sec, polymerization of ethylene in heptane, cyclohexane, methyl alcohol and acetone proceeds at a rate 10 times greater than the rate of polymerization in the gas phase at the same pressure. In carbon tetrachloride, tetrachloroalkanes of various molecular weights are formed. Initially, the rate of reaction increases but later the rate becomes constant. The rate of the process is proportional to [dose rate]$^{0.3}$, while the radiation yield is proportional to [dose rate]$^{-0.7}$. The resulting polyethylene possesses a higher density (0·945 to 0·975g/cm^3) and crystallinity than conventional high-pressure polyethylene. The tensile strength of polyethylene prepared by the radiation-chemical method differs little from that of high-pressure polyethylene.

Bagdasarian, Voevodsky, *et al.*[138] investigated graft polymerization with polytetrafluoroethylene. They employed electron spin resonance to elucidate the mechanism of the process.

Usmanov, Musaev and Tillaev[144] investigated the radiation-chemical method of preparation of graft copolymers in the systems, polystyrene-acrylonitrile and polyvinylchloride-acrylonitrile. For a polystyrene film swollen with acrylonitrile, it was observed that the reaction proceeds with doses up to 1×10^6 to 4×10^6 roentgen. The preparation of graft copolymers of polyvinylchloride and acrylonitrile was accomplished by irradiation of a polyvinylchloride film (in presence of air) followed by treatment with acrylonitrile.

As mentioned above, radiation polymerization mostly proceeds by a free-radical mechanism. However, it has recently been established that under certain conditions (e.g. at low temperatures) polymerization by irradiation can also occur by a carbonium-ion mechanism. Thus, Abkin *et al.*[139, 141] demonstrated this by copolymerizing the systems, isobutylene-vinylidene chloride, styrene-methyl methacrylate and isobutylene-styrene. Further support for the possibility that polymerization can proceed by a carbonium-ion mechanism has been obtained recently[140, 141] for acrylontrile. This molecule contains an electronegative group and is polymerized by γ-radiation from ^{60}Co in solvents which contain nucleophilic substituents (e.g. triethylamine or dimethylformamide) but is not polymerized in solvents containing an electrophilic group (e.g. ethyl chloride.). On the other hand, styrene is polymerized in ethyl

chloride. Data obtained by Abkin *et al.* for polymerization of acrylonitrile by γ-radiation in a variety of solvents at a temperature of —78°C are given in Table 2.

Table 2 *Polymerization of acrylonitrile by γ-radiation in various solvents at —78° (data of Abkin* et al.)

Monomer	Concentration (mole/l.)	Polymerization rate (10^{-5} mole $l.^{-1}$ sec^{-1}).			
		in ethyl chloride	in dimethyl-formamide	in tri-ethylamine	in bulk
Styrene	2·0	1·95	0	0	0
Acrylonitrile	3·5	0	0·97	0·67	0·21

If copolymerization of acrylonitrile and styrene in dimethylformamide solution is carried out at —78°C, the resulting copolymers are highly enriched in acrylonitrile relative to the original mixture. All these data indicate that it is possible for polymerization by radiation to occur by a carbonium-ion mechanism.

The possibility of employing ionizing radiation for the preparation of inorganic polymers is being investigated by Soviet chemists. Thus Spitsyn *et al.*[145] demonstrated the possibility that phosphonitrile chloride may be polymerized by electrons. It is of interest that the polymerization proceeds only in the presence of oxygen.

6 EFFECTS OF IONIZING RADIATIONS ON POLYMERS

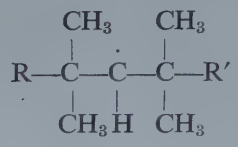
In the U.S.S.R., the first systematic investigations of the effects of radiation on polymeric materials were carried out by Kargin with collaborators[146–149] and concerned mainly the change of thermo-mechanical properties after irradiation. Further investigations in this field are being carried out by a number of workers, namely, Medvedev, Karpov, Lasurkin, Tsetlin, Slovokhotova and Taubman.

Karpov[150, 151] studied the effect of radiation on various polymers* using both α- and β-radiation from radon and the products of its disintegration, γ-radiation from ^{60}Co, fast electrons and the mixed radiation from a nuclear reactor. It was found that all polymers can be subdivided into two principal groups. Polymers in the first group are particularly susceptible to radiation destruction, e.g. rupture of the polymer chain. Polymers in which, when irradiated, cross-linking predominates, i.e., formation of chemical bonds between the macromolecules, constitute the second group.

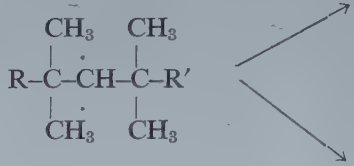
Gas evolution from polymers under irradiation was studied by Karpov *et al.*[150–152] and some conclusions were drawn concerning the mechanism of radiation-chemical reaction in polymers. For cross-linked polymers the principal gas evolved is hydrogen. Thus, for instance, irradiation of polyethylene gives a gas containing 95·5% hydrogen. This confirms the suggestion that the cross-linking process passes through an intermediate stage of formation of free radicals by the rupture of C-H bonds. Destruction of polymers is observed in all cases where the main chain of the macromolecule contains quaternary carbon atoms. Under irradiation, such polymers evolve gas which contains some hydrogen but greater amounts of other compounds which result from the cleavage of side chains at quaternary carbon atoms.

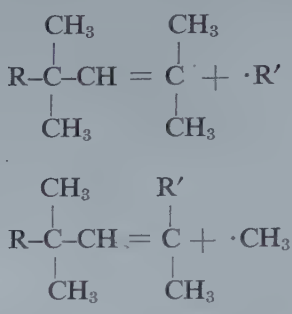
Karpov and Slovokhotova[153–155] employed spectral methods to demonstrate that irradiation of polyethylene results in the gradual disappearance of vinylidene double bonds, present in the original polymer in small amount, and the formation of new double bonds of the *trans*-vinyl type (mainly conjugated). Formation of double bonds is also observed in the case of the degraded polymers. In polyisobutylene, formation of double bonds probably proceeds by H-atom abstraction from a secondary carbon atom so that a radical of the following type results:

* Researches on the radiation chemistry of polymers were begun by Karpov in 1947.



Because of steric factors, this radical cannot cross-link and it undergoes further degradation:





Examination of infra-red spectra has helped to demonstrate that when irradiated polymers are exposed to oxygen from the air oxo groups are formed. Another phenomenon of interest observed by Karpov *et al.*[150, 152, 156] is the loss of crystallinity in polymers when exposed to γ-radiation from ^{60}Co or to fast electrons. This has been observed for polyethylene, guttapercha, the copolymer of vinyl chloride and vinylidene chloride, polytetrafluoroethylene and some other crystalline polymers.

Karpov, Blumenfeld *et al.*[157] from measurements of infra-red, ultra-violet and visible spectra and electron spin resonance spectra of irradiated polyvinylchloride have proved that the profound changes caused by electron bombardment arise from the splitting-off of hydrogen chloride and the formation of a double bond. In the opinion of the authors, free radicals are responsible for the change of colour on irradiation of polyvinylchloride. Their presence in the irradiated polymer has been detected by electron spin resonance techniques, their concentration decreasing with time. This decrease is caused both by recombination of free radicals and by interaction of the radicals with oxygen to give peroxide radicals, if oxygen is present.

The papers of Taubman *et al.*[158, 159] deal with problems of elucidating some peculiarities in the radiation degradation of polymers. They examined the effects of electron bombardment and high temperature on the degradation of polytetrafluoroethylene, polymethylmethacrylate and polyethylene. A sharp discontinuity in the rate of gas evolution caused by irradiation is observed in the narrow temperature region where melting (softening) of polymers occurs.

The phenomena of cross-linking in polymers are of interest since this process confers valuable properties on most polymers. The mechanism of cross-linking in polyethylene has been examined by Medvedev *et al.*[160, 161] who suggested that the temperature dependence of cross-linking implies that cross-linking results from the simultaneous detachment of two hydrogen atoms in a single primary act from two adjacent macromolecules.

Voevodsky *et al.*[162] examined the formation of free radicals in irradiated polyethylene by electron spin resonance methods and have detected both alkyl and allyl types. The stability of these radicals differs implying a different mechanism for their recombination. In the opinion of the authors, recombination of radicals accumulated during irradiation must play an important part in the process of cross-linking in polyethylene.

Medvedev *et al.*[163] have recently reached conclusions of considerable interest concerning the mechanism of protection by benzene rings during the radiolysis of polystyrene. This polymer is known to have a high radiation stability; formation of a single cross-link in polyethylene requires 25 to 35 eV, while for polystyrene 3000 to 5000 eV are necessary. From results of the radiolysis of toluene* labelled with deuterium the authors have concluded that the high radiation stability of polystyrene is largely caused by association of

* In the radiolysis of toluene the radicals formed have a structure similar to those formed in irradiated polystyrene.

primary radicals with cyclohexadienyl-type radicals produced in the polymer under irradiation (Figure 10).

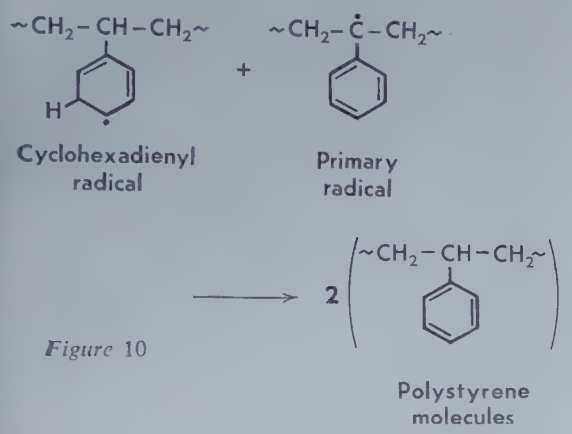


Figure 10

Lazurkin and Ushakov[164] examined the radiation cross-linking of polydimethylsiloxane. The resulting polymer possesses higher strength and resistance to freezing than those obtained by conventional methods. Radiation-chemical cross-linking (vulcanization) of resins was also investigated by Kuzminsky, Karpov and Nikitina *et al.*[165, 166] who established that rubbers with new valuable properties can be prepared.

Pavlova, Rafikov and Tsetlin[167] obtained experimental evidence that the two opposing reactions of cross-linking and of degradation by radiation proceed simultaneously for polyamides. The authors correlated the change of mechanical properties, the solution properties, the change of molecular weight and the molecular weight distribution function with conditions of irradiation. By this means they succeeded in distinguishing the concurrent reactions of cross-linking and degradation. However, cross-linking is the predominant process and vulcanization of the polyamide is the end result of irradiation of this material.

Recent investigations of the change in physical properties of polymers during their irradiation are of considerable interest. Lazurkin *et al.*[168] studied the changes in mechanical properties of different polymers during irradiation from a nuclear reactor and observed reversible effects, e.g., a change in mechanical properties induced by irradiation which disappeared when the irradiation was stopped. Reversible changes in the gas permeability of polymers during γ-irradiation were examined by Karpov *et al*[169]. When the ^{60}Co source of γ-radiation is introduced and then removed the rate of gas diffusion through the polymer undergoes a sharp increase and then returns towards the original value although remaining slightly higher.

Varshavsky, Vasiliev, Karpov, Lazurkin and Petrov[170] studied isotope exchange between deuterium gas and different solid polymers in a nuclear reactor to see whether hydrogen from the gaseous phase can be introduced into polymer molecules during irradiation. Polyethylene and polypropylene were shown to trap more hydrogen than the other polymers examined. The rate of isotope exchange is slower for polybutadiene and polystyrene. The authors have suggested that the introduction of deuterium is caused by the reaction of the polymer radicals arising from irradiation, with deuterium molecules:

$$R\cdot + D_2 \rightarrow RD + \cdot D$$
$$RH + D\cdot \rightarrow R\cdot + HD, \text{ etc.}$$

The use of isotope-exchange techniques on a wide scale will undoubtedy help to elucidate the mechanism of polymer radiolysis more completely.

7 RADIATION ELEMENTARY ACTS AND PRIMARY CHEMICAL PROCESSES

In all chemical transformations in irradiated material, secondary electrons, ions, excited molecules and free radicals (including atoms) are involved. The identification of these products is of crucial importance for elucidating the mechanism of radiation-chemical reactions. Mass spectrometry, electron spin resonance and related techniques can be used to identify these entities.

Tal'rose and Frankevich[171] employ mass spectrometry to investigate the products formed by electron impact with some simple inorganic and organic compounds in the gaseous phase and find that the ion-molecule reactions which play an important part in primary radiation-chemical processes can give excited radicals:

$$M^+ + RH \rightarrow MH^+ + \cdot R^*$$
$$M^+ + R'R'' \rightarrow (MR')^+ + (\cdot R'')^*.$$

This phenomenon is observed in many pro-

cesses in the vapour phase including:

$$H_2^+ + H_2O \rightarrow H_3O^+ + H\cdot$$
$$CH_4^+ + H_2O \rightarrow H_3O^+ + CH_3\cdot$$
$$C_3H_8 + H_2O^+ \rightarrow H_3O^+ + C_3H_7\cdot, \text{ etc.}$$

Tal'rose[172] pointed out that different types of trap can be formed by irradiation (especially of hydrocarbons) which play an important part in radiation chemistry. By irradiation of saturated hydrocarbons, unsaturated compounds and free radicals can be formed with ionization potentials lower than those of the parent molecule. These compounds and radicals can act as *p*- and *n*-traps. An increase in the number of traps in a condensed phase creates conditions which favour the primary process of recombination of 'plus-minus' pairs leaving an adjacent free valence. One of the two radicals present at the recombination centre can neutralize this free valence, the probability being practically unity for hydrocarbon systems.

The use of pulsed irradiation to measure rate constants of ion-molecule reactions is of great interest. By combining pulsed irradiation and mass spectrometric techniques, Tal'rose and Frankevich[173] determined the rate constants for the following reactions:

$$CH_4 + CH_4^+ \rightarrow CH_5^+ + \cdot CH_3$$
$$H_2O + H_2O^+ \rightarrow H_3O^+ + \cdot OH$$

At 370°K the rate constant of the former is equal to $11{\cdot}6 \times 10^{-11}$ cm^3 molecule^{-1} sec^{-1}, that of the latter is $8{\cdot}5 \times 10^{-10}$ cm^3 molecule^{-1} sec^{-1} at 410°K.

Tunitsky *et al.*[174–178] studied the dissociation of rather large molecules by electron impact using a mass spectrometer. Examination of the mass spectra of halogenated hydrocarbons[174, 175] indicated that, as the halogen content of the molecule increases, the percentage of molecular ions in the mass spectra decreases. They also found[176, 177] that the best agreement between calculated and experimental values occurs when it is postulated that there is an approximately equal probability for a fragment ion to be formed from any part of the molecule. For instance, in the mass spectrum of *n*-nonane-5-^{13}C the ions $^{13}CH_3^+$, $^{12}C^{13}CH_5^+$ and $^{12}C_2{}^{13}CH_7^+$ have been observed in amounts which are approximately equal which follows from the above assumption. From their analysis of mass spectra, the authors proposed the following scheme for the dissociation of large molecules (Figure 11).

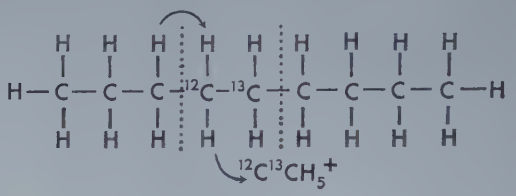


Figure 11

Quite recently Tal'rose and Frankevich[179] correlated the induced electrical conductivity with the behaviour of free radicals in irradiated solid paraffin. They measured the conductivity of paraffin irradiated at 77°K during the 'annealing-time'. In this case, an explosive increase in conductivity was discovered (Figure 12). This phenomenon is interpreted as follows. At temperatures differing by some dozens of degrees from the temperature region where 'dark' conductivity takes place, an appreciable conductivity is observed. At higher temperatures the conductivity falls to its 'dark' value. The time–temperature region for rapid increase coincides with the region where a decreasing electron spin resonance signal is observed. From these data it is reasonable to conclude that generation of ions in an irradiated solid or liquid proceeds through the formation of free radicals.

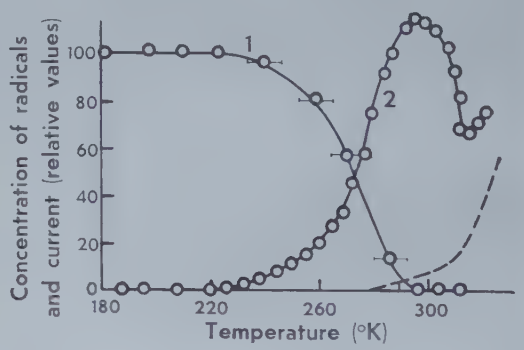


Figure 12 *Variation of radical concentration and electrical conductivity of irradiated paraffin under heating. (Heating rate equal to 22°C/min.)* (1) *concentration of radicals (in arbitrary units);* (2) *relative variation of the current through the sample at* 1,000 *V potential difference between electrodes placed inside the sample. Variation of the dark conductivity is indicated by the dotted line.*

Voevodsky *et al.*[162, 180–185] are carrying out an investigation of the radicals which appear in the radiolysis of organic and inorganic systems using an ESR technique. They have constructed an apparatus which permits the observation of ESR spectra during irradiation with fast electrons and the kinetics of radical formation have been studied[162, 180]. The radicals formed by irradiation of polytetrafluoroethylene in a vacuum at low temperatures have been found to have a long half-life. In air they are converted to peroxide radicals,

$$CF_2–\underset{\displaystyle O–O\cdot}{\underset{|}{CF}}–CF_2,$$

which can be stored at room temperature for some months.

For benzene, the radicals $C_6H_5\cdot$ and $C_6H_7\cdot$ have been found[182]:

$$C_6H_6 \rightsquigarrow C_6H_5\cdot + H\cdot;$$
$$C_6H_6 + H\cdot \rightarrow C_6H_7\cdot$$

In polyphenyls (diphenyl, *p*-ditolyl, etc.) abstraction of H atoms and CH_3 groups situated in the *para*-position to the phenyl substituent occurs to form a radical analogous to $C_6H_7\cdot$.

In this review we have not discussed some other branches of radiation chemistry at present being developed in the U.S.S.R. This paper, for instance, does not contain any description of the effect of ionizing radiation on catalysis and semi-conductors, or any discussion of chemical dosimetry or the design of radiation sources. Nevertheless, the contents of the review give an idea of the wide range of work in the field of radiation chemistry carried on in the U.S.S.R.

There is no doubt that in the immediate future more and more powerful radiation sources will become available. Elementary particle acceleration technique is improving rapidly and the power of nuclear reactors is also growing. It will soon be possible to obtain more highly active preparations of artificial radioactive isotopes. Energy released by the fission of the uranium nucleus will undoubtedly be put to practical use in the future for new chemical processes. These will open bright prospects for radiation chemistry which is one of the most progressive branches of modern physical chemistry and chemical engineering.

The exchange of scientific successes, achieved in this important field, between scientists of different countries will promote the further progress of chemical science and its applications in the interests of the whole of mankind.

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Conjugation, Hyperconjugation and Hybridization

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1 INTRODUCTION

An understanding of atomic arrangements and interatomic distances is of prime importance in considering theories of molecular structure. For the large class of aromatic compounds benzene is the key molecule to be studied and the concepts used in the modern quantum-mechanical description of its structure have also been applied to the ground-state properties of open-chain conjugated molecules such as butadiene and diacetylene. Until about 1946, the description of the bonds in these molecules concentrated mainly on the number of electrons in the bonds. Since that time it has been gradually realized that pronounced effects may also be ascribed to the different directions of carbon bonds, or different hybridizations of the carbon atoms, in various compounds.

Recently strong cases have been presented for explaining the properties of conjugated chains which take into account these directional or hybridization effects. It is the aim of this review to discuss these re-interpretations and to point out some of their implications in theories of molecular structure. In Section 2 an outline of the position up to about 1946 will be given and the developments since that time will be discussed in the later sections.

2 OUTLINE OF MOLECULAR STRUCTURE THEORIES

2.1 *Historical development*[1, 2, 3]

Molecular structure theories began with the recognition of the different combining powers of atoms. A stereochemical aspect was added to these theories after optical activity studies led van't Hoff and Le Bel to postulate a tetrahedral carbon atom in aliphatic compounds. This idea fitted the then current picture of the quadrivalence of carbon. However, for some seventy years the demonstrated chemical equivalence of the six carbon atoms of benzene and its empirical formula, C_6H_6, proved a stumbling block to structural theories. No *one* simple formula could be written, using four carbon valencies, which adequately represented the facts. Various solutions to this problem were proposed such as the two Kekulé formulae, the diagonal formula of Claus, the Armstrong-Baeyer centric formula and Thiele's theory of partial valence. It was not until the advent of quantum mechanics that the problem was finally solved in a logical way.

Meanwhile the classical electronic valence theory of Lewis and Langmuir, with its duplet and octet rules and the sharing of two electrons

in a covalent bond, was successful in focussing attention on the importance of the electrons in chemical binding. However, to explain the origin of the binding energy, quantum-mechanical calculations for the one- and two-electron bonds in H_2^+ and H_2 respectively were required.

One further valency problem still remained, namely the stereochemical one[4, 5]. What determines the direction of chemical bonds? With a carbon atom, for instance, optical spectroscopy had shown that in its ground state carbon has four valence electrons, two *s* electrons which give a spherically symmetrical electron cloud or orbital and two *p* electrons which give two dumb-bell shaped electron clouds at right angles to each other. Such an electron distribution is obviously of no use in a description of the four tetrahedrally directed bonds in methane. However, promotion of one *s* electron to the third and vacant *p* orbital (at right angles to the other two) does give four unpaired electrons and the *s* and three *p* orbitals can be combined so that four equivalent orbitals directed tetrahedrally are obtained. The carbon atom, now in its sp^3 valence state*, forms methane by pairing each one of its electrons with the electron of each hydrogen atom. These tetrahedral or sp^3 hybrid orbitals are apparently those used by carbon in aliphatic compounds.

In a similar way three orbitals in a plane and directed at 120° to each other can be made by combining one *s* and two of the three *p* orbitals. Three of the four valence electrons of carbon in aromatic compounds are in these sp^2 or trigonal hybrid orbitals and the fourth valence electron remains in a *p* orbital directed at right angles to the plane of the other three. For acetylene compounds two valence electrons are accommodated in two linear or *sp* hybrid orbitals, formed from one *s* and one *p* orbital, and the remaining two electrons are assigned one to each of the two unused *p* orbitals.

2.2 *Theory of structure of benzene*[1, 2, 3]

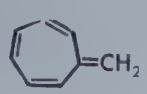
These ideas can be applied to give a quantum-mechanical description of the structure of benzene. The plane hexagonal structure of benzene requires each carbon atom to have three bonds disposed at 120° so that if two electrons are paired in each sp^2 hybrid orbital then six of the carbon valence electrons remain unused. Two simple treatments have been developed which essentially restrict themselves to a consideration of these six *p* electrons, now called π electrons, in the molecule. In the resonance theory, the six π electrons form three double bonds in one of two ways and benzene is regarded as a resonance hybrid of the two Kekulé structures. In the molecular orbital method, the six π electrons do not form three double bonds but they move in an effective field over the whole molecule and occupy in pairs the three π orbitals of lowest energy.

Though the language differs, both of these treatments predict two main effects in the ground state of benzene—firstly, complete delocalization of the π electrons, which implies the six-fold symmetry of the molecule so that all CC bonds are equivalent and intermediate in length and in character between single and double bonds, and secondly, additional thermochemical stability, measured by the resonance or delocalization energy, due to the delocalization process.

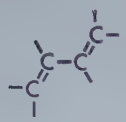
These concepts then, of resonance between equivalent structures or delocalization of π electrons, are used in the quantum mechanical solution of the problem of the benzene structure. It is relevant to point out here that although the theory explains the extra stabilizaion observed in benzene, it does *not* explain benzene's low chemical reactivity[7]. Explanation of the chemical reactivity requires a knowledge of the energy difference between the starting products and the transition state of the reaction. This differenence cannot be obtained from properties of the ground state alone[7, 8]. Theoretical delocalization energy and low chemical reactivity happen to go together in benzene but need not always do so in other molecules. Failure to recognize this has lead to the hope, often unrealized, that high π electron delocalization energy means unusually low reactivity or classical aromatic character. Heptafulvene (1) (methylene cyclohepta-

* The sp^3 valence state is not a spectroscopic state. H. H. Voge[9] has calculated that this valence state contains 31·25% of ^5S sp^3, 28·13% of ^3D sp^3, 3·125% of ^1D sp^3, 14·06% of ^3P p^4, 4·68% of ^1D p^4, 14·06% of ^3P s^2p^2 and 4·68% of ^1D s^2p^2.

triene) is a case in point. The theoretical resonance energy is 36 kcal/mole, which is about the same as for benzene, but the molecule is only stable in dilute solution, polymerizes rapidly and is very sensitive to acids, i.e. it is a thoroughly unaromatic molecule[8].



(1)



(2)

2.3 *Conjugation in acyclic molecules*

Applying these concepts to the simplest open-chain conjugated molecule, *trans*–1,3–butadiene (2), the observed shortened central C–C bond and extra stability are ascribed to the partial double-bond character of the central bond which results from some delocalization of the π electrons in the two double bonds.

The partial double-bond character of CC bonds can be derived from a calculated quantity called the 'π bond order'[1, 9]. Within the CC resonance and molecular orbital theories, the π bond order for benzene's CC bonds is found to be 1/2 and 2/3 respectively. If it is assumed that there is a continuous change in π bond order as the CC bond length changes from ethane (pure single bond 1.54 Å, with zero π bond order) through benzene (1.397 Å[10], π bond order 1/2 or 2/3) to ethylene (pure double bond 1.34 Å, π bond order 1) and to acetylene (pure triple bond 1.205 Å, π bond order 2) then the curves shown in Figure 1 can be drawn[9, 11]. With these curves the partial double-bond character of a CC bond is estimated from its observed length, e.g. in butadiene the central CC bond, with a length of 1·483 Å[12], has about 20 per cent double-bond character.

Interaction of two triple bonds across a single bond is apparently greater than that of two double bonds since the central C–C bond in diacetylene, HC≡C–C≡CH, is lowered in length to 1·38 Å[13] which indicates about 40 per cent double-bond character.

It should be noted that the concept of double-bond character is purely a theoretical one. Such a quantity can only be derived from experimental bond lengths via a theoretical model. Thus in effect the double-bond character of a bond is merely another statement of the bond length.

2.4 *Hyperconjugation in acyclic molecules*

From the observations that in propylene, $CH_2{=}CH{-}CH_3$, the C–C bond is shorter (1·501 Å)[14] than in ethane and the molecule is more stable than ethylene[3] by 3 kcal/mole, it is inferred that the three hydrogen atoms of the methyl group are able to interact across the single bond with the double bond. This interaction has been called hyperconjugation[15]. Hyperconjugation of a methyl group with a triple bond seems to be larger than with a double bond since the C–C bond length in methyl acetylene, $CH_3{-}C{\equiv}CH$, and methyl cyanide, $CH_3{-}C{\equiv}N$ is lowered to 1·46 Å[16, 17].

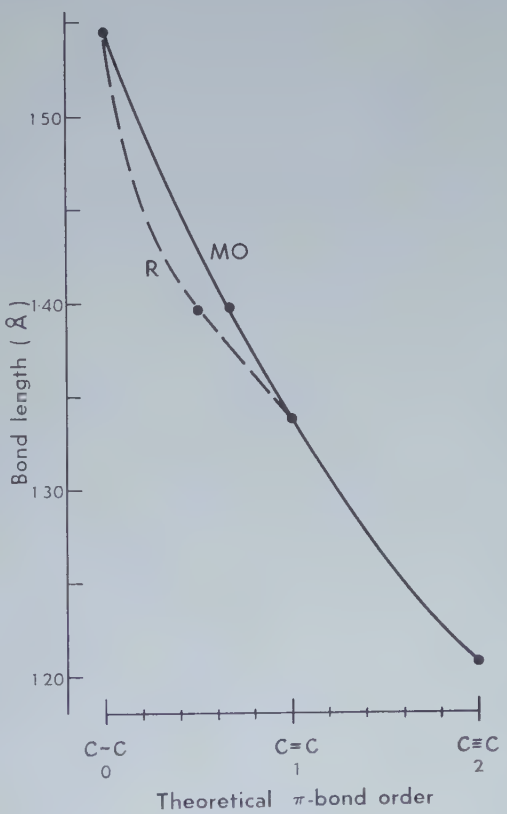


Figure 1 Relation between experimental bond length and π-bond orders of molecular orbital theory (MO) and of resonance theory (R)

2.5 *Hybridization effects*

Over the last few years it has occurred to a number of authors notably Coulson[18], Mulliken[19], and Dewar and Schmeising[20], that in calculating bond shortenings relative to the ethane C–C bond length, the effect of change of hybridization of the carbon atoms has been neglected[18, 19, 20]. In ethane the CC single bond is between two tetrahedral (sp^3) carbon atoms while in butadiene the central CC bond is between two carbon atoms in trigonal (sp^2) hybridization. Recent quantitative estimates of the effect of this change have been the bases of the arguments for[19] and against[20] conjugation and hyperconjugation in systems represented by butadiene and propylene.

3 CONJUGATION AND HYBRIDIZATION IN BUTADIENE

The two experimental facts germane to a discussion of the ground-state properties of the conjugated molecule butadiene are the length of the central C–C bond and the extra thermochemical stability of the molecule compared with an unconjugated analogue.

3.1 *Bond Length*

Trans-1,3-butadiene (2) has a planar carbon skeleton with a central C–C bond length of $1{\cdot}483 \pm 0{\cdot}01$ Å[12]. The length of this bond represents a balance between the natural length of a bond between two trigonally hybridized carbon atoms and any shortening due to double-bond character[18]. Dewar and Schmeising[20] point out that the significance attached to the observed length depends on an assessment of the relative importance of these two effects, i.e. hybridization shortening and resonance shortening. Pauling, Palmer and Springhall estimated the hybridization shortening in a =C—C bond at about 0·01 Å[21]. However, at that time the experimental uncertainties in bond lengths did not warrant the inclusion of such shortening in discussing butadiene. Later, in discussing a suggestion of Walsh's on the importance of hybridization[22], Coulson calculated a length of 1·50 Å for a pure single bond between two sp^2 carbon atoms[18], i.e. a hybridization shortening of 0·04 Å. In view of these varying estimates a more careful discussion of the observed bond lengths in butadiene and related molecules is required in an attempt to determine the natural length of the trigonal single bond.

Effect of environment on bond length

Some years ago, Herzberg and Stoicheff pointed out the dependence of bond length in a number of aliphatic molecules on the *type of adjacent bonds*[23]. A single bond adjacent to one triple bond has a length $1{\cdot}460 \pm 0{\cdot}003$ Å whereas the same bond adjacent to two triple bonds has a length of $1{\cdot}380 \pm 0.002$ Å. Moreover these distances are independent of the *type of adjacent atoms* as measured for example by electronegativity. Additional bond length data, mostly obtained by microwave spectroscopy, are incorporated in the 1959 paper of Costain and Stoicheff[24], and these with some more recent data are given in Table 1.

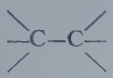
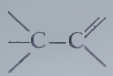
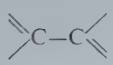
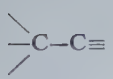
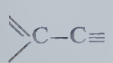
The systematic change of bond lengths with change of environment, specified by *number of adjacent atoms*[24], is shown in Figure 2. The lengths of C—C, C=C and C—H bonds fall very close to three separate straight lines. However out of a total of about 40 points there are some notable exceptions, namely the C—H bonds in methane[53] and the C—C bonds in paraffins[26–30], butadiene[12], acetyl cyanide[35] and propynal[46]. In the last two molecules, the adjacent oxygen atom may be influencing the C–C bond length. Although the paraffinic C–C bonds are noticeably shorter than 1·544 Å, this diamond C–C value is still regarded as the standard C–C bond length. The C–H length in methane and the C–D length in tetradeuteromethane have been carefully investigated by both electron diffraction and Raman spectroscopy and it seems that the divergence from CH_3 values is real[60, 61]. The butadiene

(3)

C–C bond length (1·483 Å) is anomalous not only in terms of the straight line shown in the figure but also by comparison with cyclo-octatetraene (1·462 Å)[36]. In the tub form of cyclo-octatetraene (3), there can be little interaction

between the double bonds since they are almost at right angles to each other and hence the butadiene length should be equal to or shorter than 1·46 Å.

Table 1 Carbon–carbon and carbon–hydrogen bond lengths

Length (Å)	Method*	Molecule	Reference
			
1·5447	X	diamond	25
1·543	IR	ethane	26
1·534	IR	ethane	27
1·526	M	propane	28
1·525	M	isobutane	29
1·533 ± 0·003	ED	*n*-butane, *n*-pentane, *n*-hexane, *n*-heptane	30
			
1·501	M	propylene	14
1·505	ED	isobutylene	31
1·501	M	acetaldehyde	32
1·503	M	acetyl fluoride	33
1·499	M	acetyl chloride	34
1·490	M	acetyl cyanide	35
			
1·483	ED	butadiene	12
1·462	ED	cyclo-octatetraene	36
			
1·459	M	methyl acetylene	16, 17
1·458	M	methyl cyanide	16, 17
1·457	X	dimethylacetylene	37
1·464	M + ED	trifluoromethyl acetylene	38
1·455	M	methyl trifluoromethyl acetylene	39
1·458	M	methyl chloroacetylene	40
1·458	M	propargyl chloride	41
1·46	M	trifluoromethyl cyanide	42
1·460	M	trichloromethyl cyanide	43
1·460	M	*t*-butyl cyanide	44
1·456	M	methyl diacetylene	45
1·454	M	methyl cyanoacetylene	45
			
1·466	M	acetyl cyanide	35
1·446	M	propynal	46
1·426	M	vinyl cyanide	24
≡C–C≡			
1·382	M	cyanoacetylene	47
1·375	M	methyl diacetylene	45

Length (Å)	Method*	Molecule	Reference
≡C–C≡			
1·377	ED	dimethyl diacetylene	48
1·376	R	diacetylene	13
1·380	R	cyanogen	49
>C=C<			
1·339	R	ethylene	50
1·339	M	vinyl cyanide	24
1·337	IR	ethylene	51
1·334	ED	ethylene	52
>C=C=			
1·313	M, R	ketene	53, 54
1·309	R	allene	54
=C=C=			
1·284	R	butatriene	55
1·279	ED	carbon suboxide	56
–C≡C–			
1·208	M	methyl cyanoacetylene	45
1·205	M	methyl acetylene	16, 17
1·205	IR	acetylene	57
1·205	M	cyanoacetylene	47
1·205	ED	dimethyl diacetylene	48
>C–H			
1·105	M	methyl acetylene	16, 17
1·103	M	methyl cyanide	16, 17
1·102	IR	ethane	26
1·092	R	methane	58
=C–H			
1·086	M	vinyl cyanide	24
1·084	R, ED	ethylene	50, 51, 52
≡C–H			
1·063	M	hydrogen cyanide	59
1·059	IR	acetylene	57
1·057	M	cyanoacetylene	47
1·056	M	methyl acetylene	16, 17

* X = X-ray diffraction, ED = electron diffraction, IR = infra-red spectroscopy, M = microwave spectroscopy, R = Raman spectroscopy.

Hybridization interpretation of bond lengths

The empirical regularities shown in Figure 2 can be interpreted in terms of the hybridization of the bonding carbon atoms. Bernstein[62] had earlier pointed out that C–C lengths are well represented by

$$l = 1{\cdot}544 - 0{\cdot}42\ (6 - n_1 - n_2) \quad \textbf{(1)}$$

where sp^{n_1} and sp^{n_2} are the states of hybridiza-

tion of the bonding carbon atoms. The quantity $n_1 + n_2$ is the same as the number of atoms adjacent to the bond, so that a C–C bond with five neighbours is an sp^3-sp^2 bond whilst the bond with four neighbours can be of either sp^2-sp^2 or sp^3-sp type. This interpretation determines the natural lengths (Å) of the various bonds as 1·544, sp^3-sp^3; 1·502, sp^3-sp^2; 1·460, sp^2-sp^2 and sp-sp^3; 1·418, sp^2-sp; 1·376, sp-sp.

Whilst there seems to be a real deviation from the C–C straight line for butadiene and some of the sp-sp^2 bonds, it is true that the C–C bond length changes equally in going from sp^3-sp^3 to sp^3-sp and from sp^3-sp to sp-sp, or in other words the bond length depends on the s/p ratio in the hybrid orbitals. If now one plots the observed lengths against the mean fraction (or mean percentage) of s orbital in the hybrid orbitals, the distribution of points is as shown in Figure 3. This mean fraction, f, defined as $\frac{1}{2}(1 + n_1)^{-1} + \frac{1}{2}(1 + n_2)^{-1}$, is the measure of s character used by Dewar and Schmeising[63]. The C–C lengths are now well represented by

$$l = 1{\cdot}544 - 0{\cdot}672\ (f - 0{\cdot}25) \qquad (2)$$

and the natural lengths (Å) of the various bonds become 1·544, sp^3-sp^3; 1·516, sp^3-sp^2; 1·488 sp^2-sp^2; 1·460, sp-sp^3; 1·432, sp-sp^2; 1·376, sp-sp. In this plot the butadiene point lies closer to the line but cyclo-octatetraene and the sp^3-sp^2 points are farther away. The position with respect to the sp-sp^2 bonds is not much changed.

M. G. Brown has also considered the variations in CC bond lengths[64]. From a larger number of C–C bond lengths (some 219), of lower accuracy than those included in Figure 2 and 3, he obtained the following mean bond lengths (Å) for the various bond types (the figures in brackets indicate the number of the total sample within ± 0·01 Å of the mean): sp^3-sp^3 1·544 (40/51), sp^3-sp^2 1·510 (31/67), sp^2-sp^2 1·466 (16/37), sp^3-sp 1·456 (34/37), sp^2-sp 1·432 (3/10), sp-sp 1·374 (14/17). He has justified his hybridization classification of the mean bond lengths by observing that the sp^3-sp^3, sp^2-sp^2 and sp-sp lengths give a straight line when plotted against the square of the hybridization parameter, λ, which defines the extent of mixing of the $2s$ and $2p$ wave functions in the hybrid orbitals. One of the sp^3 tetrahedral orbitals is (unnormalized) $s+\sqrt{3}p$, i.e. $\lambda^2=3$, one of the sp^2 trigonal orbitals is $s+\sqrt{2}p$, i.e. $\lambda^2=2$ and one of the sp diagonal orbitals is $s+p$, i.e. $\lambda^2=1$.

Mikhailov[65] considered C–C bond lengths on the basis that they are determined solely by the hybridization of the carbon atoms. Using the bond lengths observed in methyl acetylene ($\diagdown$C–C≡, 1·460 Å) and benzonitrile ($\diagup$C–C≡, 1·419 Å) he derives the same natural bond lengths as Bernstein.

These sets of natural C–C bond lengths fall into two groups differing only in the measure used to characterize the hybrid orbitals. The Bernstein, Costain-Stoicheff, Brown group obtains a linear relationship with the quantity $n_1 + n_2$, which can be interpreted as (a) the number of p orbitals in the hybrid or (b) the number of adjacent atoms or (c) $\lambda_1^2 + \lambda_2^2$. Dewar and Schmeising obtain a linear relationship with the quantity $\frac{1}{2}[1+n_1) + (1+n_2)^{-1}]$ interpreted as the mean fraction of s orbital in the hybrid. Which set is chosen seems largely a matter of personal preference.

As Dewar and Schmeising[20, 63] point out, these estimates suggest that most of the shortening of the central bond in butadiene is due to the change of hybridization of the carbon atoms from sp^3 in ethane to sp^2 in butadiene. However this hybridization interpretation of the natural bond lengths implies either that the carbon covalent radius is different at the carbon and hydrogen sides of its bonds if hydrogen has a constant covalent radius or that the hydrogen covalent radius varies from 0·320 to 0·371 Å if the carbon covalent radius is the same in the C–C and C–H bonds. One of these implications is necessary to explain the different slopes of the C–C and C–H lines in Figures 2 and 3.

Resonance and hybridization interpretation of bond lengths

An alternative interpretation of the bond lengths shown in Figures 2 and 3 is obtained by using the C–H bond lengths to estimate the natural lengths of the various C–C bonds and then by ascribing any extra shortening to partial double-bond character due to

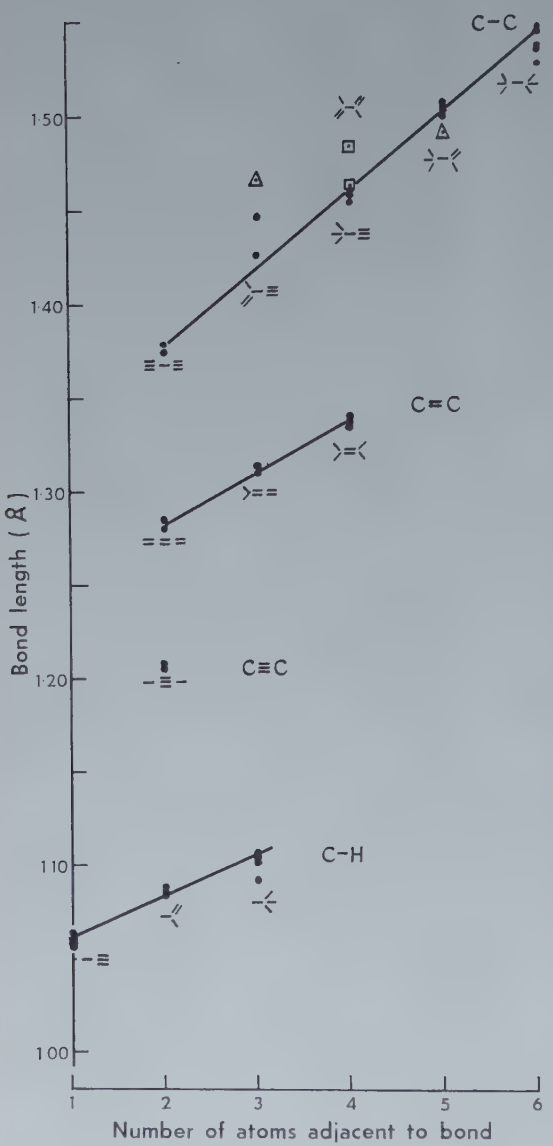


Figure 2 CC and CH bond lengths plotted against the number of atoms adjacent to the bond. The points △ are the two C–C bonds of acetyl cyanide and the points □ are the C–C bonds of butadiene and cyclo-octatetraene

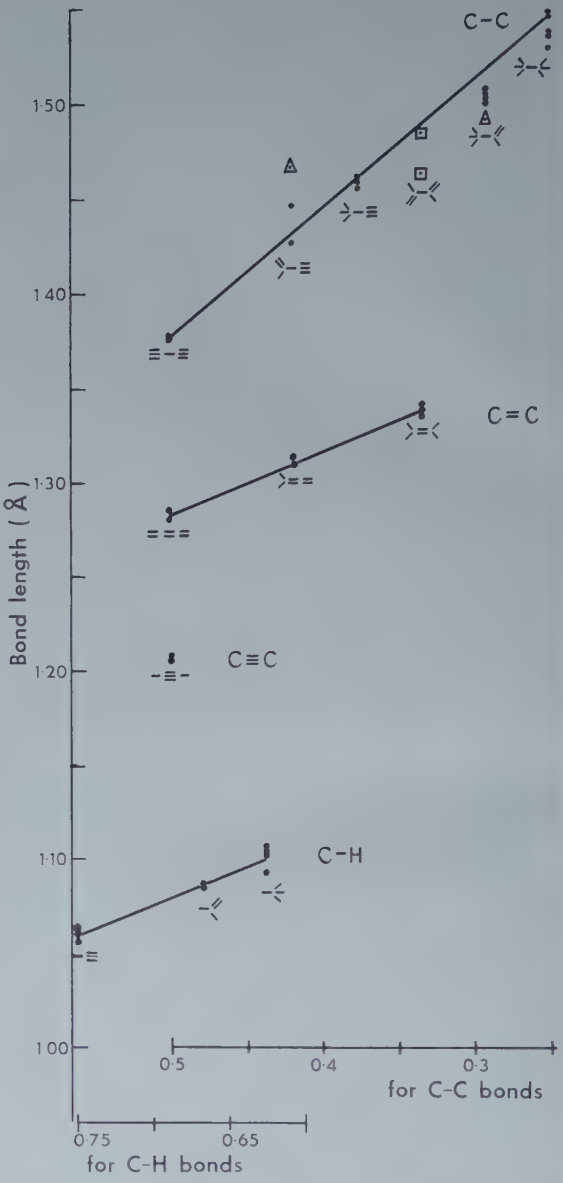


Figure 3 CC and CH bond lengths plotted against the mean fraction of s-character of the bonding orbitals. The points △ are the two C–C bonds of acetyl cyanide and the points □ are the C–C bonds of butadiene and cyclo-octatetraene

double- or triple-bond conjugation or CH_3 hyperconjugation[18]. Assuming that the C atom has the same covalent radius when bonding to another C or to an H atom, and using the diamond covalent radius, 0·772 Å, the H covalent radius derived from methane or ethane and the C–H distances in ethylene and acetylene, the natural lengths of C–C bonds are as shown in Table 2. The resonance shortening of the C–C bond in butadiene is then 0.027 Å (1·510 — 1·483) and this shortening is used to estimate the double-bond character of that bond.

Table 2 C–C Natural bond lengths

Hybridization classification of C–C bond type	Calculated Natural C–C bond length (Å)	
sp^3-sp^3	1·544*	1·544†
sp^3-sp^2	1·527	1·537
sp^2-sp^2	1·510	1·530
sp^3-sp	1·501	1·511
sp^2-sp	1·484	1·504
sp-sp	1·458	1·478

* These lengths are derived using $r_H = 0{\cdot}330$ Å from the ethane C–H bond length.

† These lengths are derived using $r_H = 0{\cdot}320$ Å from the methane C–H bond length.

Theoretical estimates of natural sp^2-sp^2 C–C bond length

It is difficult to decide between the two interpretations given above of the experimental bond length, and therefore various theoretical estimates of the natural length of the trigonal single bond have been made in an attempt to push the decision one way or the other.

Coulson's estimate[18]. Coulson reasoned that the distance from the origin of the centroid of charge of the hybrid orbital should be proportional to the covalent radius of the C atom. He derived a formula for the covalent radius in terms of the hybridization parameter, λ. This method of estimation, which has been used recently by Bak and Hansen-Nygaard[66], leads to a bond length essentially the same as that derived from the C–H bond length under the assumption of a constant H covalent radius.

Dewar and Schmeising's estimate[63]. These authors have offered the linearity of their bond-length-bond — order plot as evidence for a length of 1·488 Å for a pure single bond between sp^2 C atoms. The bond lengths for benzene, graphite and ethylene are plotted against the unnormalized molecular orbital π bond orders calculated with the inclusion of overlap. These bond orders are unnormalized in the sense that the bond order of ethylene $(1 + S)^{-1}$, is 0·80 when S, the overlap integral, is equal to ¼. However, other π bond order definitions are possible and these do not give the same bond length at zero π bond order. Three other quantities (Coulson, Mulliken and Pauling π bond orders[67]) are plotted in Figure 4. On the basis of theoretical arguments, it has been suggested that the appropriate bond orders to use when overlap is taken into account are the Mulliken bond orders[67], and it should be noted that this line extrapolates to the same bond length as the Dewar-Schmeising line.

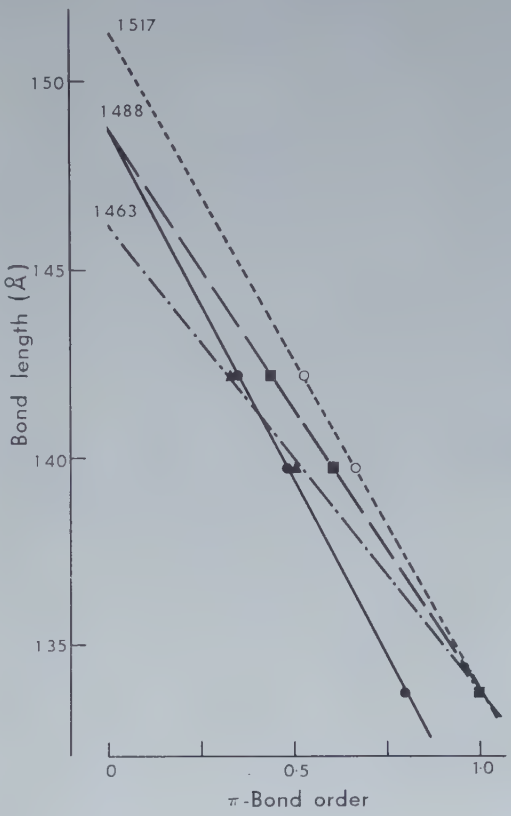
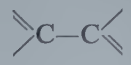


Figure 4 Linear bond length, π-bond order relationships using ethylene, benzene and graphite bond lengths and the Coulson bond orders ○, the Mulliken bond orders ■, the unnormalized Mulliken bond orders as used by Dewar and Schmeising ● and the Pauling bond orders ▲. The figures adjacent to the lines give the extrapolated value of the bond length for zero π-bond order

Pauling's estimate[68]. In the molecular orbital treatment of the ethylenic double bond two equivalent descriptions can be used, namely, a double bond of two bent single bonds ('banana' bonds) or one trigonal single (σ) bond together with a weaker bond between two π orbitals(σ-π or 'sausage' bonds). These two descriptions are not equivalent in the valence-bond treatment and Pauling considers

it an advantage to discuss the double bond in terms of two bent single bonds. Such a description means that the single-bond—double-bond angle should be 125°16′ as against 120° for the sausage bond description. Experimentally many bond angles in double-bonded compounds are closer to 125° than to 120°, but in ethylene itself the angle is 121°30′. Using the bent bond picture, Pauling points out that the double distortion in the system  might be expected to lower the C–C bond length. From a consideration of a number of saturated and unsaturated molecules he concludes that the bending caused by one double bond should contract a C–C bond by 0·02 Å while a triple bond should cause a contraction of 0·04 Å. With the sp^2-sp^2 length of 1·504 Å, butadiene has a central bond with about 15 per cent double-bond character.

Mulliken's estimate[19]. Mulliken, in advocating both hybridization and resonance shortenings in reply to Dewar and Schmeising, has recognized the difficulty of assessing the changes caused by hybridization, and he has reversed the usual argument. He suggests that present day calculations give reliable estimates of π bond orders so that the resonance shortening is first calculated. The π bond orders for the central bond in butadiene, calculated by various theoretical methods, seem to converge to a value of about 0·20, which corresponds to a resonance shortening of about 0·03 Å. With the observed butadiene C–C length of 1·483 ± <0·01 Å, the natural length of the trigonal bond is then 1·51 ± 0·01 Å.

The various theoretical and experimental estimates of the natural CC single bond lengths are collected in Table 3. The trigonal bond lengths group themselves into two regions, firstly 1·46-1·49 Å and secondly 1·51-1·53 Å. In deriving the values in the second group, the existence of both hybridization and resonance shortenings is recognized whereas the values in the first group are derived on the basis that all bond-length effects are due to hybridization changes only.

3.2 *Resonance energy*

The heat of hydrogenation of butadiene indicates an extra stability of 8·63 kcal/mole at 0°K relative to two moles of ethylene or 3·91 kcal/mole relative to 1-butene[63].

The usual calculation of resonance or delocalization energy would compare butadiene with 1-butene as a reference compound[3] but in the latter molecule, the central bond is between sp^3 and sp^2 carbon atoms whereas the central bond in butadiene is of the sp^2-sp^2 type. The previous discussion indicates that bond lengths vary with hybridization and so also should bond energies. No allowance for the difference in bond energies of these two bonds is usually made. For this reason Dewar

Table 3 C–C Natural bond lengths

Method of estimation	Bond length (Å) of type					
	sp^3-sp^3	sp^3-sp^2	sp^3-sp	sp^2-sp^2	sp^2-sp	sp-sp
Bernstein[62], Costan-Stoicheff[24] . .	1·544	1·502	1·460	1·460	1·418	1·376
Brown[64]:						
experimental means	1·544	1·510	1·456	1·466	1·432	1·374
from derived covalent radii . . .	1·544	1·505	1·459	1·466	1·420	1·374
Mikhailov[65]	1·54	1·50	1·46	1·46	1·42	1·38
Dewar-Schmeising[63]	1·544	1·516	1·460	1·488	1·432	1·376
Pauling, Springhall and Palmer[21] .	1·54	1·53	1·52	1·52	1·51	1·50
Using constant r_H = 0·320 Å . .	1·544	1·537	1·511	1·530	1·504	1·478
= 0·330 Å . .	1·544	1·527	1·501	1·510	1·484	1·458
Coulson[18], Bak and Hansen-Nygard[66]	1·544	1·531	1·505	1·517	1·492	1·467
Mulliken[19]	1·54	1·51	1·485	1·51	—	1·45
Pauling[68]	1·54	1·52	1·50	1·50	1·48	1·46

and Schmeising suggest that comparisons should always be made with simple molecules such as ethylene when double bonds are involved, and that the value so obtained should be termed the 'stabilization energy'[20].

This stabilization energy still contains a contribution which represents the energy change due to the hybridization change during the hydrogenation. Dewar and Schmeising point out that it is necessary to evaluate this quantity before any interpretation of stabilization energies can be made. To do this they set up a bond-length—bond-energy relationship[63] whose consistency they check by comparing the calculated and observed heats of formation for a number of hydrocarbons. Agreement is generally better than 0·1 per cent except in the case of conjugated dienes (2 kcal/mole difference) and aromatics (28 kcal/mole difference). In this derivation they take care of the known deviations from additivity of the energies of paraffins by distinguishing primary and secondary C–H bond energies.

Dewar and Schmeising obtain the hybridization energy from the set of equations **(3-6)**[63]. In these equations, X is the heat of hydrogenation of ethylene, RE the true resonance energy of butadiene, E_{33} and E_{22} are the bond energies of sp^3-sp^3 and sp^2-sp^2 CC single bonds while ϵ_{p3}, ϵ_{s3} are the C–H bond energies with tetrahedrally hybridized primary and secondary carbon atoms and ϵ_2 is the C–H bond energy with a trigonally hybridized carbon atom. The numerical values of these quantities are shown in Table 4.

The numerical value of the hybridization energy, E_h, is 6·24 kcal/mole so that the resonance energy of butadiene is only 2·39 kcal/mole. This quantity now represents the stabilization due to any delocalization of π electrons and is referred to classical butadiene with localized electrons and with proper allowance made for the more energetic sp^2-sp^2 C–C bond in that classical molecule. This value is very sensitive to the length and hence the strength of the trigonal C–C bond: 1·489 Å gives a resonance energy of 2·39 kcal whereas 1·479 Å lowers the energy to essentially zero. Thus the resonance energy of butadiene is less than one quarter of the observed stabilization energy and is probably even smaller in view of the uncertainty surrounding the length of the trigonal single bond.

Table 4 *Bond energies and bond lengths of Dewar and Schmeising*[63]

Type of bond		Bond length (Å)	Bond energy Symbol	Bond energy Value (kcal/mole) at 0°K
C–C	sp^3-sp^3	1·544	E_{33}	84·56
	sp^2-sp^2	1·489	E_{22}	94·99
C–H	sp^3 (primary)	1·102	ϵ_{p3}	97·01
	sp^3 (sec, tert)	1·107	ϵ_{s3}	96·30
	sp^2	1·086	ϵ_2	100·52

Mulliken, in replying to this analysis of Dewar and Schmeising, points out that it is not necessary to assume that bond energies do not change with hybridization[19]. A formal system of equations can be written down which, with some plausible assumptions, effectively reduce to those used for the usual resonance energy calculation. A full discussion along

$$2CH_2{=}CH_2 + 2H_2 \rightarrow 2C_2H_6 + 2X \qquad (3)$$

$$CH_2{=}CH{-}CH{=}CH_2 + H_2 \rightarrow 2CH_2{=}CH_2 - E_{22} - E_{HH} + 2\,\epsilon_2 - RE \qquad (4)$$

$$2CH_3 - CH_3 \rightarrow C_4H_{10} + H_2 + E_{33} + E_{HH} + 4\,\epsilon_{s3} - 6\,\epsilon_{p3} \qquad (5)$$

Adding equations (**3**), (**4**) and (**5**) gives

$$CH_2{=}CH{-}CH{=}CH_2 + H_2 \rightarrow C_4H_{10} + 2X + E_{33} - E_{22} + 4\,\epsilon_{s3} - 6\,\epsilon_{p3} + 2\,\epsilon_2 - RE$$

The stabilization energy of butadiene referred to ethylene is defined as the heat of hydrogenation of ethylene minus the heat of hydrogenation of butadiene. Thus

$$\begin{aligned} \text{stabilization energy} &= 2X - \{2X + E_{33} - E_{22} - 6\,\epsilon_{p3} + 4\,\epsilon_{p3} + 2\,\epsilon_2 - RE\} \\ &= E_{22} - E_{33} + 6\,\epsilon_{p3} - 4\,\epsilon_{s3} - 2\,\epsilon_2 + RE \\ &= E_h + RE \end{aligned} \qquad (6)$$

these lines does not seem to have been given.*

There are thus two interpretations of the ground-state properties of butadiene: one allows for about 20 per cent double-bond character in the central bond, while the other explains both the bond length and the stabilization energy as due to changes of hybridization. This latter picture denies the need for the resonance effects, i.e. butadiene in its ground state is very well represented by its classical formula, $CH_2=CH-CH=CH_2$. This representation is not intended to apply to chemical reactions where a transition state is also involved nor to spectral properties where an excited state is also involved.

Here are two viewpoints and it does not seem possible to make a clearcut test between them, but the recognition of hybridization effects means that there is a logical flaw in the molecular orbital bond-order versus bond-length curve shown in Figure 1. The curve there is made by joining points corresponding to different hybridization types and should be replaced by a family of curves, one for each type of hybridization[18, 64]. If bond shortenings are explained in terms of hybridization and resonance, then the single, double and triple bonds of *sp-sp* type would be represented on one curve by diacetylene, butatriene and acetylene respectively while the sp^2-sp^2 bonds of butadiene, benzene and ethylene would be on another curve, and sp^2-*sp* bonds on a third curve. These three curves are shown in Figure 5. For the other types of bonds, only a small portion of the curve close to the appropriate single bond length would be meaningful. If the bond shortenings are explained in terms of hybridization only, then many C–C bonds formerly regarded as partial double bonds should be reclassified as single bonds.

4 HYPERCONJUGATION AND HYBRIDIZATION IN PROPYLENE

The C–C distance in propylene is $1{\cdot}501 \pm 0{\cdot}004$ Å[14] while the heat of hydrogenation at 0°K is 3·09 kcal/mole less than that of ethylene[63].

In the light of the discussion of butadiene, there are two interpretations of this bond distance. If the 'hybridization only' approach is

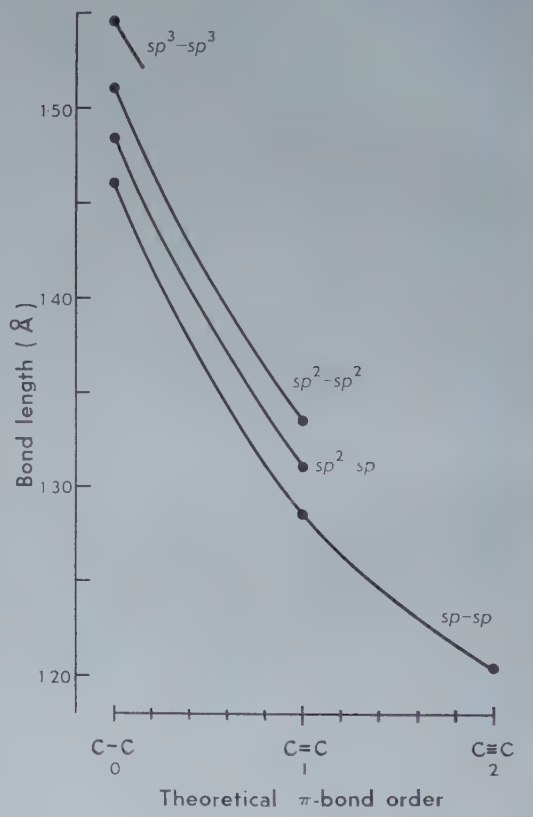


Figure 5 Relation between bond length and π-bond order for sp^2-sp^2, sp^2-sp *and* sp-sp *CC bonds after the single bond lengths have been corrected for hybridization effects*

* Cyclo-octatetraene should also be considered in this connection as well as butadiene and a number of authors[19, 69] have commented on some of the difficulties associated with this cyclic and non-resonating molecule. Mulliken[19] has noted that although conjugation is destroyed when double bonds are almost perpendicular to each other, hyperconjugation is possible and so some stabilization is still expected. On the other hand Skinner and Turner have pointed out that since cyclo-octatetraene contains four trigonal single bonds, its stabilization energy on the Dewar and Schmeising interpretation should be four times that of butadiene[69]. Yates has concluded, from thermochemical evidence for these two molecules, that there is significant stabilization energy in butadiene which cannot be attributed solely to a pure trigonal single bond and that the trigonal C–C bond energy is higher in butadiene than in cyclo-octatetraene due to changes in conformation between the sp^2 carbon atoms[69]. At this stage the significance of the evidence on these points is not clear.

adopted, then the bond length is the one expected for a sp^3-sp^2 single bond. There can be no hyperconjugation interaction in this case. On the hybridization and resonance approach, the natural length for this bond is about 1·52 Å so that the further shortening of about 0·02 Å corresponds to about 10 per cent double-bond character due to hyperconjugation.

The apparent stabilization energy is interpreted as mostly hybridization energy on one hand and as resonance energy on the other hand. Dewar and Schmeising, using their bond energy values and bond lengths, calculate the hybridization energy due to the more energetic sp^3-sp^2 bond as 2·93 kcal/mole leaving only 0·15 kcal/mole for possible interpretation as resonance energy[63].

There are problems associated with the view that propylene contains the representative sp^3-sp^2 bond. Figures 2 and 3 predict its length to be 1·502 Å and 1·516 Å respectively. The experimental points for other molecules with sp^3-sp^2 bonds show rather a large scatter, but none of the accurate microwave bond lengths are as high as 1·516 Å. But the hybridization energy has been evaluated with this high bond length, and a shorter bond length would increase E_h so that the resonance energy would become negative. It is possible that this result would be modified too if the sp^3-sp^3 distance were lowered to 1·530 Å, as seems a distinct possibility from the latest C–C bond lengths in paraffinic hydrocarbons[63, 26–30].

5 CONJUGATION AND HYBRIDIZATION IN BENZENE

As outlined in the introduction, the ideas of double-bond character and resonance energy were originally introduced in discussing the ground-state properties of benzene[1, 2, 3]. Now if the Dewar and Schmeising 'hybridization only' viewpoint of conjugated chains is accepted, what modification does this entail for the theory of benzene in particular and aromatic molecules in general? The answer is, only a slight modification.

5.1 *Bond length*

The experimental bond length is 1·397 Å[10], which if entered on Figures 2 and 3 falls between the C–C and C=C sp^2-sp^2 bond lengths. The concept of the π bond order still retains its validity but the bond-order versus bond-length curve for sp^2-sp^2 bonds should be used and here some doubt has been introduced as to the appropriate length for zero π bond order (1·46, 1·49, 1·51 or 1·54 Å). A fuller discussion of the implications of this revision will be found in Section 6.

5.2 *Resonance energy*

For benzene the relevant experimental heats of hydrogenation are as follows.[63]

At 0°K

$3C_2H_4 + 3H_2 \rightarrow 3C_2H_6 + 93{\cdot}12$ kcal/mole,

$C_6H_6 + 3H_2 \rightarrow C_6H_{12} + 44{\cdot}01$ kcal/mole.

Stabilization energy referred to ethylene = 49·11 kcal/mole.

Traditionally the resonance energy of benzene is defined as the difference in energy between the actual molecule and a hypothetical Kekulé structure or cyclohexatriene[3]. Formerly the energy of cyclohexatriene was calculated from the energy of three molecules of cyclohexene. However, following the considerations outlined above for butadiene, it should be noted that cyclohexene has sp^3-sp^2 bonds that are absent in benzene. Dewar and Schmeising therefore picture cyclohexatriene as having double bonds 1·338 Å long and single bonds 1·489 Å long, and then allow for the stabilizing effect of the three =C—C= bonds[63]. This effect is $3E_h$ = 18·72 kcal which gives a conjugation energy of 49·11-18·72 = 30·39 kcal/mole. This conjugation energy is lowered to 24·33 kcal/mole if the sp^2-sp^2 carbon bond length is as low as 1·479 Å. However it is important to allow for the difference in σ bond energy between cyclohexatriene and benzene. It takes more energy (Dewar and Schmeising estimate about 15 kcal/mole more) to compress three single bonds (1·489 Å) to the length 1·338 Å than to compress six such bonds to the benzene bond length (1·397 Å). The actual energy due to the delocalization process is now about 15 kcal/mole, or 9 kcal/mole with the lower trigonal single-bond length and energy. Dewar and Schmeising believe therefore that the true benzene resonance energy is only

about one quarter of the observed stabilization energy.

Thus little has been changed—the benzene bonds are still regarded as intermediate between single and double bonds and the reinterpretation of the stabilization energy does not make the stabilization any less real. Also it is not Dewar and Schmeising's intention to add support to those who would deny the reality of resonance or delocalization in benzene and so describe it as cyclohexatriene[70].

6 BOND LENGTH RELATIONSHIPS

One sure conclusion from the previous discussion is that the single-bond length used in evaluating the double-bond character of aromatic bonds should be lower than the ethane value. Just how much lower seems to be a matter of opinion. A large number of bond-length—bond-character curves have been proposed and this review seems an appropriate place to compare them and also to discuss them in the light of a lower single-bond length.

The measures of double-bond character that have been most often used fall into two main groups, each group arising from an approximate theoretical treatment of aromatic molecules. One group is derived from valence-bond theory and the other from molecular-orbital theory. Ham and Ruedenberg[67] further classified the molecular-orbital quantities into two types, *bond orders* which are based on bond contributions to the π electron energy, e.g. the Coulson and Mulliken bond orders, and *bond populations* which are based on a geometrical division among bonds of the total π electronic charge. It was also found that the resonance theory bond orders, or the Pauling bond orders based on counting Kekulé structures only, could be given a simple definition in the molecular-orbital theories[67, 71]. Pair-wise comparisons between the bond orders and bond populations of the molecular-orbital theory and the Pauling bond orders have shown good correlation between these quantities[67, 72]. There seem to be much poorer correlations between the molecular-orbital and valence-bond quantities.

The numerous bond-length—bond-order curves are summarized in Table 5. For the bond populations only linear relationships have been used and these are not included in the table[67, 73]. Some of the tabulated relationships have been justified theoretically but others have been simply judged by their ability to pass through the ethylene, benzene and graphite points. In some of the theoretical justifications the equation

$$K_{12}(1-p)/p = (l-d)/(s-l) \qquad (7)$$

is obtained, where p is the bond order of the bond whose length is l[9, 66, 74]. In principle s, d and K_{12} are adjustable parameters. Usually the double-bond length, d, is chosen as 1·34 Å as in ethylene leaving two constants open to choice namely, s, the single bond length, and K_{12} the ratio of the single-bond stretching force constant to the double-bond constant. Then either s or K_{12} is chosen and the other determined by requiring the curve to pass through the benzene point. A linear relationship is obtained if $K_{12} = 1$. Most of the relationships can be conveniently classified by their s and K_{12} values, as in Table 5; some other bond-order—bond-length curves of other forms are listed at the end of the table.

The success of any one of these theoretical curves is particularly sensitive to the experimental precision of the bond lengths as well as to the choice of the parameters of the curves. Until recently the uncertainty in bond lengths determined from X-ray diffraction methods was ± 0·02 Å. However, appreciably better X-ray bond lengths of naphthalene[72], anthracene[72] and chrysene[88] have recently become available as a result of more accurate refinement processes and Raman spectral determinations of the bond lengths in benzene[10] and ethylene[50] are also available.

In view of the wide variety in the choice of parameters of the theoretical curves shown in Table 5 and the doubt surrounding the value of s, it seemed worthwhile to survey some of these measures of double-bond character without specific prejudice towards one or other theoretical form. The survey method chosen was to fit a straight line to the points by minimizing the perpendicular distance of the points from the line. This procedure is justified by the view that since aromatic CC bond lengths are usually 1·40 ± 0·06 Å, i.e. a 5 per cent variation, the bond length changes can be regarded as perturba-

Table 5 Summary of bond-order—bond-length relationships

The table gives the values of the parameters s and d (in Å) and K_{12}. A brief description of the procedure used to determine the three parameters is also given. Note that $K_{12} = 1$ gives a straight line.

1 *Using Coulson bond orders* (p^c)

Coulson[9]. $s = 1{\cdot}54$ and with $d = 1{\cdot}33$, $K_{12} = 0{\cdot}765$ is determined by acetylene.

Coulson[18]. s calculated as 1·50 and $K_{12} \approx 1$ with ethylene ($d = 1{\cdot}34$) benzene and graphite.

Longuet-Higgins and Salem[75]. With ethylene ($d = 1{\cdot}35$) benzene and graphite, then $K_{12} = 1{\cdot}0$ and $s = 1{\cdot}50$.

M. G. Brown[64]. $s = 1{\cdot}46$, $d = 1{\cdot}335$ and $K_{12} = 1{\cdot}0$.

Goodwin and Vance[76]. $s = 1{\cdot}50$ and $K_{12} \approx 1$ with ethylene ($d = 1{\cdot}34$) benzene and graphite.

This review, Figure 4. With ethylene ($d = 1{\cdot}337$), benzene and graphite, then $K_{12} = 1{\cdot}0$ and $s = 1{\cdot}513$.

2 *Using self-consistent Coulson bond orders*

Pritchard and Sumner[77]. $s = 1{\cdot}54$ and $K_{12} = 0{\cdot}765$ with $d = 1{\cdot}33$.

Bak and Hansen-Nygaard[60]. s calculated as 1·517 and $K_{12} \approx 1$ with ethylene, d calculated as 1·35, benzene and butadiene.

3 *Using Coulson bond orders calculated with configuration interaction*

Pariser[78]. $s = 1{\cdot}54$ and $K_{12} = 0{\cdot}765$ with $d = 1{\cdot}33$.

4 *Using Mulliken bond orders* (p^m)

This review, Figure 4. With ethylene ($d = 1{\cdot}337$), benzene and graphite, then $K_{12} = 1$ and $s = 1{\cdot}488$.

Dewar and Schmeising[63] using $p^m(1 + S)^{-1}$ where S is the overlap integral. With ethylene ($d = 1{\cdot}34$) benzene and graphite, then $K_{12} = 1$ and $s = 1{\cdot}488$.

5 *Using Pauling bond orders (Kekulé structures only)* (p^p)

Pauling and Brockway[11]. $s = 1{\cdot}54$ and with ethylene ($d = 1{\cdot}34$), benzene and graphite, $K_{12} = 0{\cdot}333$.

Bernstein[79]. $K_{12} = 1{\cdot}0$ and with ethylene ($d = 1{\cdot}34$) and benzene, then $s = 1{\cdot}46$.

Pauling[74]. $s = 1{\cdot}504$ and with ethylene ($d = 1{\cdot}334$) benzene and graphite, then $K_{12} = 0{\cdot}544$.

Bak and Hansen-Nygaard[60]. s calculated as 1·517, d calculated as 1·350 and with benzene, then $K_{12} = 0{\cdot}393$.

Cruickshank and Sparks[72]. With ethylene (1·337), benzene and graphite, $K_{12} = 0{\cdot}75$ and $s = 1{\cdot}477$.

This review, Figure 4. With ethylene ($d = 1{\cdot}337$), benzene and graphite, and $K_{12} = 1{\cdot}0$, then $s = 1{\cdot}462$.

6 *Using Valence-Bond Theory bond orders*

Penney 'vector model' method[80]. Curve drawn through ethane, graphite, benzene and ethylene. It can be represented by $s = 1{\cdot}54$ and $K_{12} = 0{\cdot}615$ with $d = 1{\cdot}33$.

Vroelant and Daudel 'spin states' method[81]. Curve drawn through butadiene, benzene and ethylene. It can be represented by $s = 1{\cdot}46$ and $K_{12} = 0{\cdot}64$ with $d = 1{\cdot}34$.

Miscellaneous Relations

Bernstein[82]. $l = s[\frac{2}{3} + \frac{1}{3}(\frac{1}{3})^{\frac{1}{2}p^c}]$. s determined as 1·55 by acetylene.

Kavanau[83]. $l = 1{\cdot}015 + 0{\cdot}875\ (\frac{2}{3})^p$ uses Penney bond orders, constants determined by ethane, ethylene and acetylene.

Gordy[84]. $p^c = 6{\cdot}80l^{-2} - 1{\cdot}71$ uses bond orders which include hyperconjugation[85].

Deas[86]. $l = 1{\cdot}40 + (2{\cdot}27\ p^2 - 17{\cdot}38p + 9{\cdot}70)/(176{\cdot}08p^2 + 186{\cdot}08p + 100)$ for $0.4 \leqslant p \leqslant 1{\cdot}0$. Bond orders are derived from Hall's standard excited state approximation[87]. They turn out to be numerically equal to the Coulson bond orders p^c.

tions from an equibond-length molecule. Then to a first approximation a straight line relationship between bond length and the measure of the perturbation might be expected.

The bond orders of Coulson, the self-consistent Coulson bond orders of Pritchard and Sumner, the bond orders of Mulliken, Pauling and the 'spin states' method together with the LCAO and free-electron bond populations for the bonds of ethylene, benzene, naphthalene, anthracene and chrysene are shown in Figure 6. The orthogonal least-squares straight lines are also shown. The lines fit the bond lengths fairly well, with the Pauling bond orders a little better correlated than the other quantities. The bond lengths for zero π bond order range from 1·447 to 1·547 Å and so add nothing new to the estimates for the trigonal single-bond length.

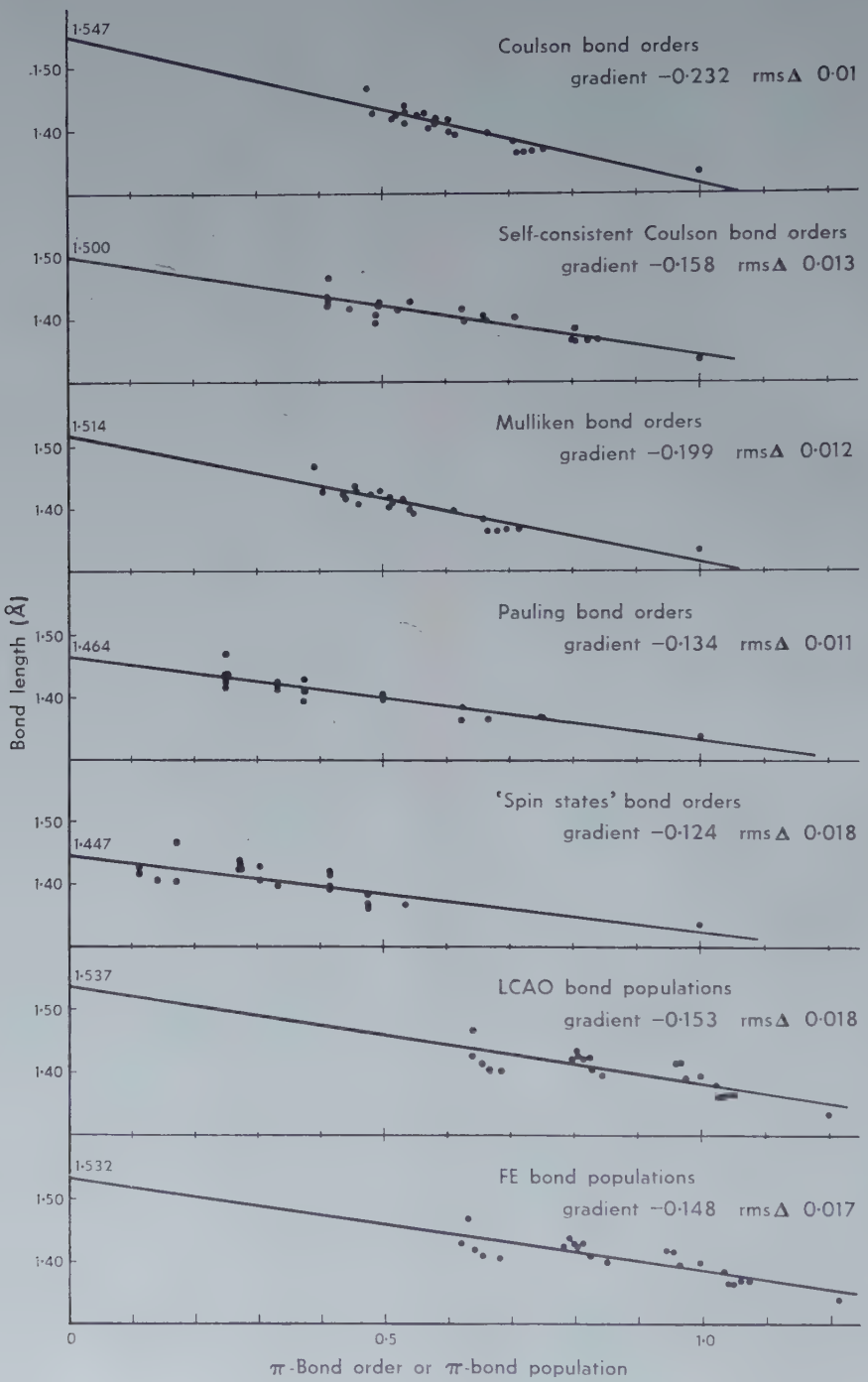


Figure 6 Plot of bond length versus π-bond orders and π-bond populations for ethylene, benzene, naphthalene, anthracene and chrysene. The straight lines are determined by minimizing the orthogonal deviations of the points. The gradients of the lines and the resulting root mean square deviations (rms Δ) of the points from the lines are also shown. The figures adjacent to the lines give the calculated bond length for zero bond order or bond population

7 SUMMARY AND CONCLUSION

Unfortunately the present discussion has not been able to decide between the two interpretations of the effects on a single bond of adjacent multiple bonds and methyl groups in acyclic compounds. One interpretation ascribes all the effects to hybridization changes, and provided the different types of C–C bonds are differentiated, the single classical formula for one of these acyclic compounds gives a good description of its ground-state properties. In the other interpretation the hybridization and delocalization effects are of comparable magnitude. However, regardless of the interpretation used, the discussion does point out that hybridization plays a more important role in determining ground state properties than has been recognized hitherto.

For aromatic molecules, where two classical formulae can be written, the concept of delocalization is required in both interpretations.

Finally it should be noted that, as the two descriptions of ethylene show, hybridization is to a certain extent an artificial phenomenon. Its general use is necessary to preserve the concept of a localized bond containing two electrons and most of the authors discussing these problems have used it for lack of a better model. It may be possible to explain the experimental facts in terms of some other theoretical model. Perhaps the observed dependence of bond lengths on the number of adjacent atoms should be expressed in terms of nonbonded van der Waals interactions between adjacent groups along the lines suggested by Bartell[89].

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REVIEWS OF PURE AND APPLIED CHEMISTRY

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This journal contains review and general articles on all aspects of pure and applied chemistry. Manuscripts offered for publication will be welcome, and authors are asked to send a statement of the scope of the proposed article to the editor before submitting the manuscript.

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Synthesis of Purines from Pyrimidines

by J. H. LISTER, Ph.D.,
(*Chester Beatty Research Institute, Institute of Cancer Research, London*)

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3 SYNTHESES FROM 4,5-DIAMINOPYRIMIDINES (THE TRAUBE SYNTHESIS)
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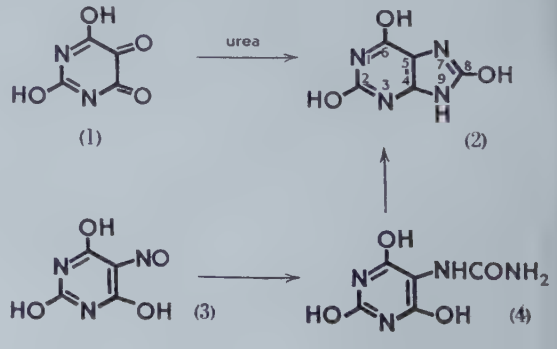
1 INTRODUCTION

Purine chemistry has undergone two distinct periods of development of which the first, covering the final twenty years of the last century and the first few years of the present one, produced the classical synthetic procedures described below. During the next thirty years few contributions were made to this field until the nature and purpose of the nucleic acids in cell metabolism were appreciated. From these studies came a revival of interest in purine and pyrimidine chemistry which continues today, mainly by workers seeking heterocyclic bases with antimetabolic properties for use in the chemotherapy of neoplastic diseases. The scope of this second period of development, in which the classical methods were elaborated by utilizing modern techniques, is indicated by the hundreds of new purines and purine analogues which have been made in the last twenty years.

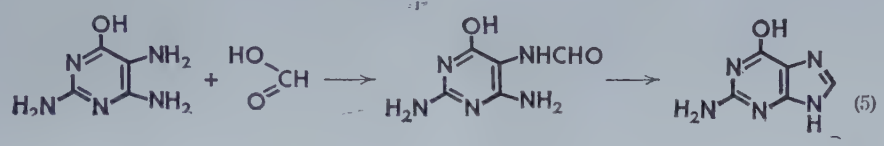
This review is directed towards the available routes for the synthesis of purines from pyrimidines, but for a more general survey of purine chemistry attention is drawn to an excellent chapter which appears in the *Chemistry of Carbon Compounds* Vol. IVc[1], whilst specialized topics are dealt with in the report of the Symposium on the *Chemistry and Biology of Purines*[2]. For a detailed historical background the earlier reviews of Fischer[3] and Biltz[4] should be consulted.

2 HISTORICAL

The first naturally occurring purine to be obtained in a pure state was uric acid (2,6,8-trihydroxypurine) (2) isolated, in 1776, by Scheele from gall stones. The much disputed structure was finally settled over a hundred years later by Emil Fischer's unambiguous synthesis[5] in 1895. Previously the acid had been obtained on a few occasions, for example, by condensing alloxan (1)[6] with urea, but none of these preparations afforded a complete structural confirmation. Fischer's synthesis consisted of reducing 5-nitrosobarbituric acid (3) to the amine and converting this with potassium cyanate into the ureido derivative (4). To cyclize this to uric acid (2) he originally used molten oxalic acid but this was replaced in a subsequent preparation by refluxing mineral acid.

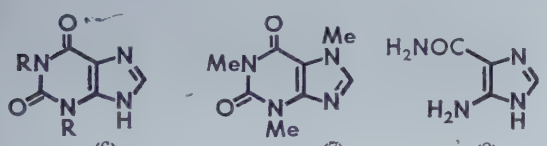


These two procedures were superseded shortly afterwards by a more versatile approach devised by Traube[7] who utilized the condensation of a 4,5-diaminopyrimidine with a simple carbonyl compound such as formic acid. In essence the reaction was a two-stage one in which the product of the first stage, a 4-amino-5-formamidopyrimidine, was then cyclized by suitable treatment to the purine.



(5)

In this way, the first direct synthesis of guanine[7] (5) was achieved and there quickly followed similar syntheses of other naturally occurring purines such as xanthine (2,6-dihydroxypurine) (6; R = H), theophylline (1,3-dimethylxanthine) (6; R = Me) and caffeine (1,3,7-trimethylxanthine) (7)[8].



(6) (7) (8)

In subsequent preparations cyclizing agents other than formic acid were used and these are dealt with in the section devoted to the Traube synthesis (3.1 *et seq.*).

The routes outlined above, which were developed before the turn of the century, form the basis of those in general use today. At this point, however, mention must be made of a more recent and fundamentally different route which is outside the scope of this review but is of growing importance. It utilizes imidazole derivatives of the type (8) as starting material and proceeds in a reverse manner to the methods outlined above, in that the pyrimidine moiety is formed last. In this respect it follows the biosynthetic pathway by which purine nucleotides are formed in the cell. The elegant *in vivo* work of Buchanan and his colleagues[9] on purine metabolism has shown that imidazole ribose phosphates are precursors of adenine, guanine and hypoxanthine nucleotides.

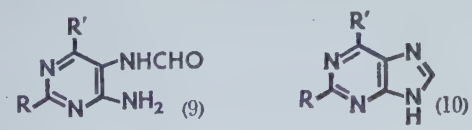
3 SYNTHESES FROM 4,5-DIAMINOPYRIMIDINES (TRAUBE SYNTHESIS)

One example of this reaction, the synthesis of guanine, is given above, but formic acid represents only one of the wide range of reagents available for cyclizing purposes. Carboxylic acids, thioacids, amides, ureas and thioureas, are the ones usually employed but a number of other compounds has been used.

Choice of the most suitable reagent depends primarily on the substituent required at the 8-position of the final product, but the possible action of the reagent on the substituents at the 2- and 6-position has also to be taken into consideration. The 4,5-diaminopyrimidines may be monosubstituted on either of the amino groups and cyclization then leads to a 7- or 9-substituted purine.

3.1 *Cyclizations with carboxylic acids (and formamide)*

Until recently formic acid was the reagent of choice for the preparation of purines unsubstituted at the 8-position. In a minority of cases isolation of the intermediate 5-formamido derivatives (9) is not necessary as prolonged heating under reflux gives the purines directly; in this way 6-hydroxy-2-methyl-(10; R = Me, R′ = OH)[10], 2-mercapto-(10; R = SH, R′ = H,[11] 2-amino-6-hydroxy-(10; R = NH_2, R′ = OH),[12] and hypoxanthine (10; R = H, R′ = OH)[12] have been obtained. Usually heating with formic acid produces only the intermediate formyl derivative (9) and to effect ring closure a further step entailing the removal of the elements of water is required.



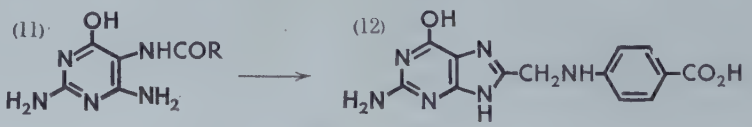
(9) (10)

The simplest means of dehydration is by heating the formamidopyrimidine (9) to above its melting point[11]. This method has been widely

used for making 9-substituted[13] and also a few 7-substituted purines[14], in some cases the fusion being carried out in an inert atmosphere[15]. Sublimation is useful for preparing volatile products, for example 9-methyl purine[16], whilst distillation is of more limited application for the preparation of purines of low melting point. It was by this last method that Isay[17] and later workers[15] directly synthesized purine (10; R = R′ = H) itself.

Some strongly acidic 5-formamidopyrimidines, notably hydroxy and amino hydroxy derivatives, are not themselves readily cyclized by heating but this difficulty can be overcome by fusion of their sodium salts[10,18]. Alternatively, the reaction can be carried out in solution[10,14] by heating the pyrimidine with aqueous alkali and one case is reported[19] where this method was utilized for the large scale preparation of the purine (10; R = SMe, R′ = NMe_2), giving a much superior yield to the other cyclizing procedures tried.

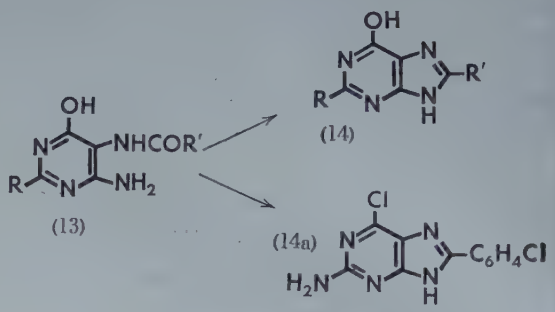
The use of inorganic reagents for dehydration purposes has been successful but limited. Thus, sulphuric acid was used[20] to cyclize the acylated pyrimidine (11; R = $CH_2NHC_6H_4$-CO_2H *(p)*) to the purine analogue (12) of pteroic acid. A more widely used reagent is



Reagent H_2SO_4

phosphoryl chloride which, when mild conditions are employed, gives the 8-methylpurines (14; R = Me, OH and NH_2, R′ = Me) from the corresponding 5-acetamidopyrimidines[21] (13; R = Me, OH and NH_2, R′ = Me) and has proved successful when fusion of the sodium salts failed to give purines[22]. Under more forcing conditions the phosphoryl chloride acts both as a dehydrating and chlorinating agent and it is possible to convert in one step a 6-hydroxypyrimidine (13; R = NH_2, R′ = pC_6H_4Cl) to a 6-chloropurine[22] (14a). A mixture of phosphoryl chloride and diethylaniline can also be used for this purpose[23]. Corresponding 6-bromopurines are obtained when phosphoryl bromide is used[22].

Phosphoryl halides are not suitable for the cyclization of nuclear *N*-alkylated formamidopyrimidines as examples are known in which both *N*(1)-methyl[24] and *N*(3)-methyl derivatives[25] have been cyclized but demethylated in the process.



Reagent $POCl_3$

The formylating power of formic acid can be increased by the addition of acetic anhydride which produces *in situ* acetic formic anhydride. Although this has been used to formylate amines[26] it has had little application so far to purine synthesis. One example is recorded, that of 2-methoxypurine[15] (10; R = OMe, R′ = H), in which it was used to prepare the crude formyl derivative of the diamino pyrimidine which was then cyclized to the purine by sublimation. Formic acid did not formylate the pyrimidine.

When thermal dehydration in solution is required, amides such as formamide and high boiling cyclic tertiary amines, like pyridine and quinoline, are found to be the most suitable solvents[19]. A mixture of *N*-formyl morpholine and formic acid has been used to prepare 2-aminopurine[15] (10; R = NH_2, R′ = H) from 2,4,5-triaminopyrimidine and likewise adenine (10; R = H, R′ = NH_2) (see ref. 29) from 4,5,6-triaminopyrimidine.

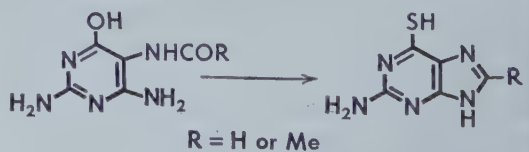
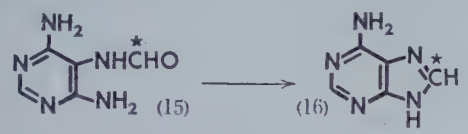


Figure 1 Reagents, P_2S_5/pyridine

By using pyridine containing phosphorus pentasulphide thiation of a hydroxyl group

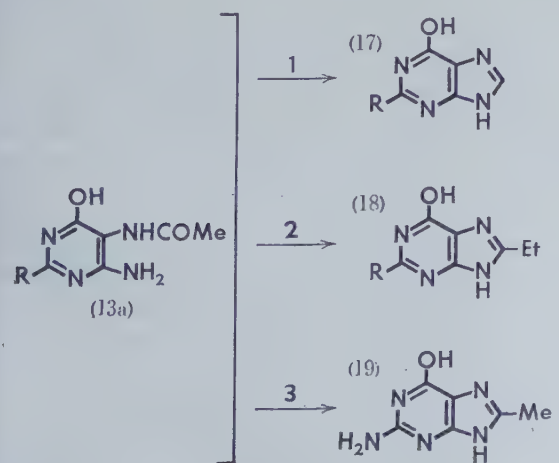
concomitant with cyclization can be accomplished and by this route an elegant conversion of 6-hydroxy pyrimidines to 6-mercaptopurines is achieved[27] in an overall yield of 50% (Figure 1). This method avoids the attendant risks involved in cyclizing 6-mercaptopyrimidines (see below).

One serious disadvantage associated with the use of formamide is its thermal instability near the boiling point. Dissociation occurs according to the equation, $HCONH_2 \rightleftharpoons CO + NH_3$, and the solvent becomes a strong formylating medium. This was shown when 4,6-diamino-5-formamidopyrimidine (15), labelled with ^{13}C in the formyl group, was cyclized to[28] adenine (16).



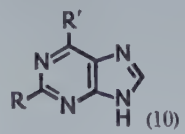
The product was found to contain only 25% labelled adenine due to exchange between the formyl and formamide carbon atoms. A similar result was obtained by another group[29] using both formamide and dimethylformamide, but when the higher boiling formyl morpholine was employed, adenine containing 80% of radio-active purine was obtained.

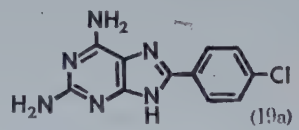
Interchange of radicals between solvent and acylaminopyrimidine is found to be fairly common with this type of cyclization. Where the acyl group of the solvent amide is different from that of the pyrimidine the resulting 8-alkylpurine is found to be derived from the amide used.



Reagents 1, $HCONH_2$; 2, $EtCONH_2$; 3, $MeCONH_2$

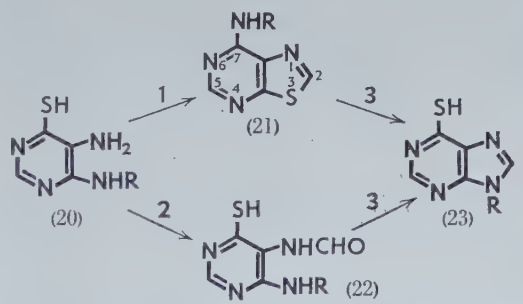
Many examples of this type of interchange are known, thus formamide closure of the pyrimidines (13a; R = Me, OH and NH_2) affords the 8-unsubstituted purines (17; R = Me, OH and NH_2)[21] whilst the use of propionamide[30] gives the corresponding 8-ethyl purines (18).





Aryl as well as acyl groups are also affected, and this is shown by the large amount of 2,6-diaminopurine (10; R = R′ = NH_2) obtained as byproduct when 5-4′-chlorobenzamido-2,4,6-triaminopyrimidine[22] is cyclized to the purine (19a). Where such interchanges are likely, the 'matching' of acyl groups is desirable as in the use of acetamide to prepare the 8-methyl purine[27] (19).

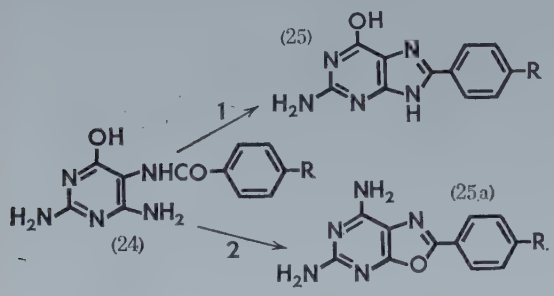
Due to the spacial equivalence of the 4- and 6-positions of the pyrimidine ring the 6-substituent in a 4,5-diaminopyrimidine can also become involved in the cyclization. Numerous examples are known of competitive cyclizations occurring[31,32] with 6-mercaptopyrimidines (20) in which (5,4-*d*) thiazolopyrimidines (21) are preferentially formed.



Reagents 1, *conc.* HCO_2H *at high temp.*; 2, *dil.* HCO_2H *at low temp.*; 3, *alkali or formamide*

One group of workers[31] was able to define the conditions for this reaction and show that concentrated formic acid and high temperatures favour thiazolopyrimidine formation. However, these derivatives are easily transformed[32] into the isomeric mercaptopurines by heating with aqueous alkali or formamide; it is presumed that the reaction proceeds by way of the intermediate (22). An analogous type of cycli-

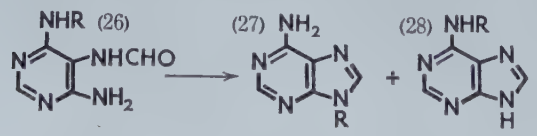
zation in which a 6-hydroxyl group participates has been noted[22] when old samples of phosphoryl chloride were used to cyclize 4-amino-5-benzamidopyrimidines of the type (24) to 8-phenylpurines (25).



Reagents 1, *fresh* $POCl_3$; 2, *old* $POCl_3$

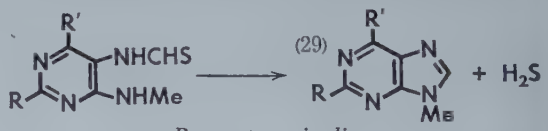
Substantial amounts of the oxazolo (5,4-*d*) pyrimidine (25a) were isolated from the reaction mixture, but by using freshly distilled phosphoryl chloride and anhydrous conditions only negligible amounts of the byproduct were obtained. It seems possible, therefore, that, as in thiazolopyrimidine formation, the presence of strong acid may predispose towards this type of cyclization.

If the 4,5-diaminopyrimidine is substituted at the 6-position by a secondary amine then invariably this is the group that is involved to the greater extent, leading to the formation of a 9-substituted purine (27). Any of the isomeric 6-substituted amino purine (28) can be removed by digestion of the product in aqueous alkali[33] in which it is soluble by virtue of the acidic hydrogen on the imidazole nitrogen.



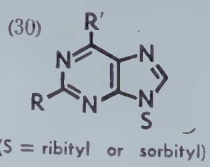
Halogeno-pyrimidines cannot be converted to the corresponding purines with formic acid because hydrolysis of the halogen occurs in all cases and hydroxypurines are obtained[34].

Dithioformic acid (HCS_2H) and its salts have been widely employed in the Traube synthesis of purine nucleosides. Their use followed from the observation that aqueous dithioformate solutions readily thioformylate 5-aminopyrimidines[35] even in the cold and that such derivatives on melting gave the purine by loss of hydrogen sulphide. Later workers found that heating the thioformamidopyrimidine in water or anhydrous tertiary bases[36] was sufficient to afford the purines. This mild treatment, in contrast to the more drastic methods of cyclization normally adopted, offered more hope of successfully cyclizing sugar-containing pyrimidines in that it reduced the risk of fission of the glycosidic linkage. A number of 9-methyl purines *e.g.* (29; R = OH, R′ = Me; R = MeS, R′ = NH_2) were first prepared as model compounds by this route and none of the 6-isomer was obtained. The method was then extended to the preparation of 9-glycosyl purines using 5-amino-4-glycosylaminopyrimidines and here, in contrast to the model compounds, a mixture of 6- and 9-isomers did result[37] when the xylosyl derivative was used. On the other hand, only the 9-isomer resulted with the corresponding ribosyl



Reagent quinoline

pyrimidine. As with the formyl derivatives cyclizations can be brought about in pyridine, quinoline or alkali (usually sodium methoxide)[38]. The 9-D-ribityl and 9-D-sorbityl derivatives of adenine (30; R = H, R′ = NH_2) and guanine (30; R = NH_2, R′ = OH)[39] are readily obtained by classical methods because



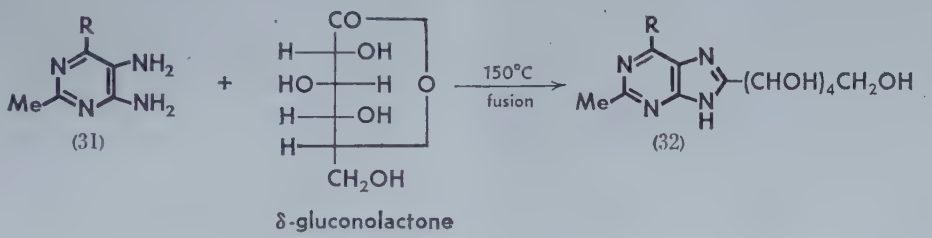
(S = ribityl or sorbityl)

reduction of the sugar has stabilized the glycosidic linkage. In these cases formylation followed by aqueous alkali or formamide can be safely used to close the ring.

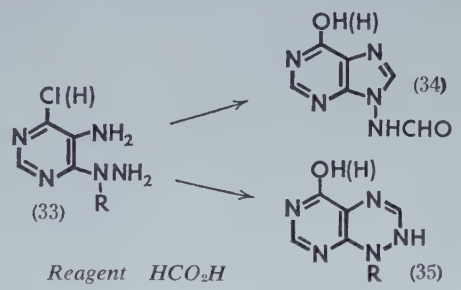
Direct condensation of sugars with 4,5-diaminopyrimidines gives 5-glycosylaminopyrimidines and attempts to cyclize these to 7-glycosylpurines[40] generally result in loss of the sugar residue. More successful is the fusion of sugar lactones with 4,5-diaminopyrimidines[41] which leads directly to 8-glucitylpurines. Thus, the δ-gluconolactone with the pyrimidines (31; R = NEt_2 or morpholyl), for example, gives the products (32; R = NEt_2 or morpholyl).

Although formic and thioformic acids have been the most extensively used of the aliphatic acids, other homologues[42] including some unsaturated long chain acids[43] and dicarboxylic acids[10] have been utilized to prepare 8-alkyl substituted purines. In a few examples acid chlorides were used instead[15,22,23,44]. Some points of interest have arisen from the use of

The excellent formylating properties of formamide (see refs. 28 and 29) make it a valuable cyclizing agent which has largely replaced formic acid in the synthesis of 8-unsubstituted purines. Formylation and ring closure occur simultaneously and the product is isolated by cooling after dilution of the formamide solution with water. Because the overall effect is



oxalic[10] and glycollic acids[41,45] with 4,5-diaminopyrimidines. Usually condensation of 1,2-dicarbonyl (or potential dicarbonyl) compounds with 4,5-diaminopyrimidines follows the Isay reaction[46] and gives rise to pteridines, but in each of the three examples in Figure 2 only one of the carbonyl groups takes part in the reaction and purines are obtained. The expected pteridines are given in parentheses.



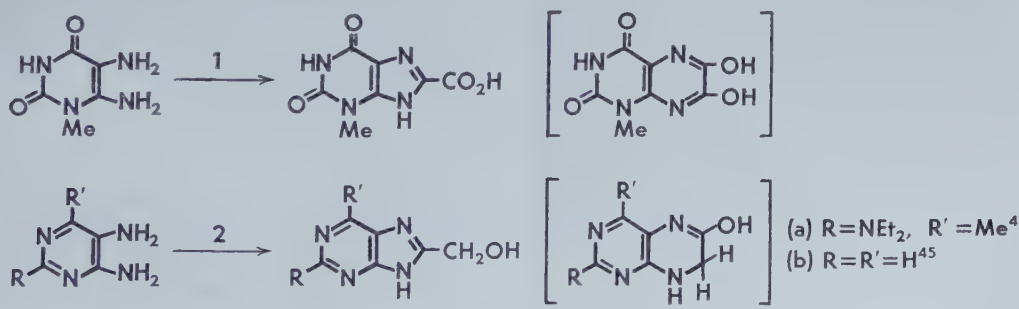


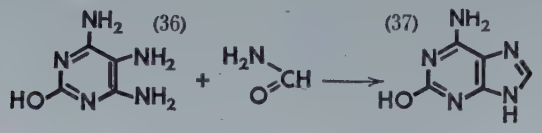
Figure 2
Reagents 1, *oxalic acid;* 2, *glycollic acid*

A further example in which cyclization produces five- rather than six-membered rings is given by the 5-amino-4-hydrazinopyrimidines (33)[47]. The pyrimidine (33; R = H) with an excess of formic acid gives the 9-formamidopurine (34) but if the α-nitrogen of the hydrazino group be substituted as in (33; R = alkyl) then cyclization to the pyrimidotriazine (35) follows.

the same as with using formic acid it is not inappropriate therefore to include such cyclizations in this section. The remarks made earlier on the risks of alternative cyclizations due to interaction with mercapto and secondary amino-groups apply equally well to this reagent.

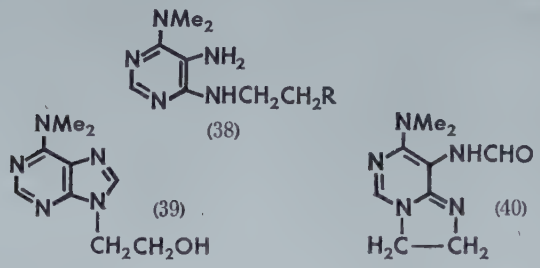
The first application was to the synthesis of isoguanine[48] (37) when 4,5,6-triamino-2-

hydroxypyrimidine (36) was heated with a mixture of formamide and formic acid. This method was successful whereas previous attempts at cyclization with a mixture of formic acid and sodium formate had failed. Later it



Reagent HCO_2H *or mineral acid*

was discovered that, in practice, formic acid could be omitted especially if a mineral acid or the salt of the pyrimidine (usually sulphate or hydrochloride) was used. With the free base alone cyclizations has often proved[12] not successful. This method has since been extensively used to prepare purines of widely differing characters[19,33,49–53].

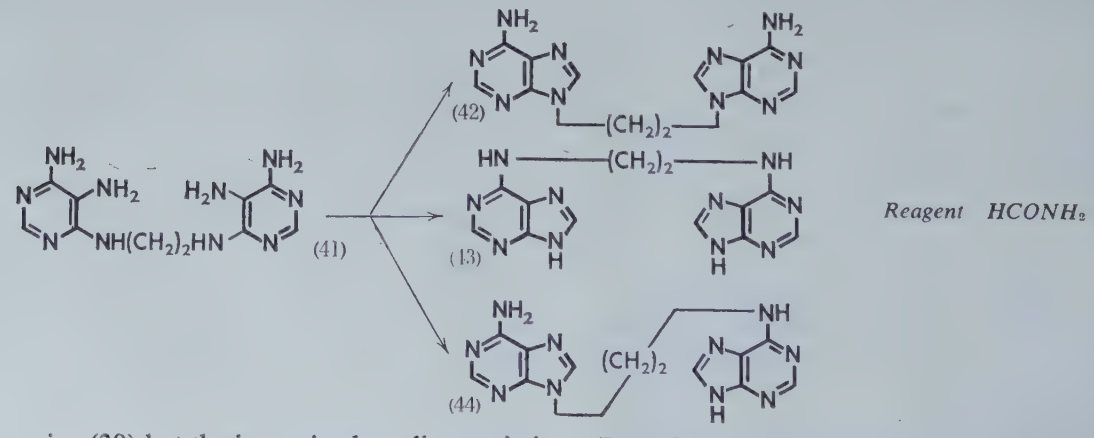


Usually formamide ring closures proceed smoothly but side reactions can occur. When 4-2′-hydroxyethylpyrimidine (38; R = OH) is used in the presence of a small amount of hydrochloric acid, the product is not the purine (39) but the isomeric glyoxalino pyrimidine (40)[54]. This could only result from chlorination of the hydroxyl group by the acid present to (38; R = Cl) which then undergoes subsequent cyclization on to a ring nitrogen before formylation and ring closure to the purine can occur. Cyclizations of this type have been studied[55,56] and the conditions under which they take place are known fairly precisely.

As with formic acid the presence of a secondary amino group leads to competitive modes of cyclization, but almost invariably the 9-substituted isomer predominates. One interesting illustration of this is the cyclization of the 1,2-bis(4,5-diamino-6-pyrimidinyl)ethane (41) which gives three products[57]. The main one is the 9-linked adenine (42) which comprises 80% of the total yield. It is surprising, however, to find that the second largest component is the asymmetrically linked isomer (44) (18%) rather than the expected 6-purinylamino derivative (43) which is present only in trace amounts (1 to 2%).

Procedures which enable more than one stage to be carried out in the same reaction medium are most valuable. One such synthesis in which a nitrosopyrimidine is reduced, formylated and ring closed in a mixture of formic acid and formamide[58,59] has now been extended so that the previous stage, nitrosation, can also be included[60].

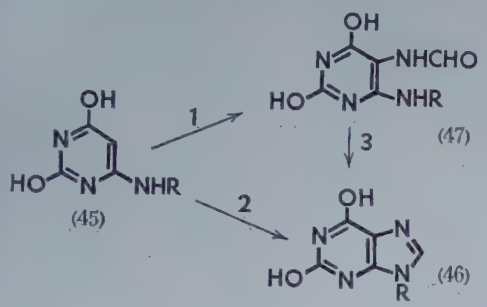
By this route a 6-alkylaminouracil of the type (45; R = Me, Et or $CH_2C_6H_5$) is nitrosated in the acid-amide mixture by the addition of sodium nitrite, reduced to the amine with sodium hydrosulphite or zinc dust and finally cyclized by vigorous heating to the purine (46;



Reagent $HCONH_2$

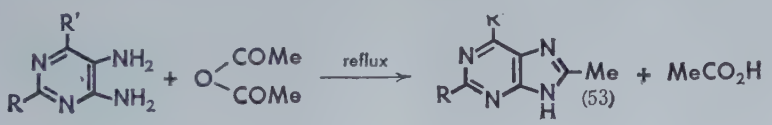
R = Me, Et or $CH_2C_6H_5$). If milder conditions are employed at the final stage the intermediate formyl derivative, typified by (47), is obtained[59,61].

A novel cyclization is reported[62] using a mixture of *NN*-dimethylformamide and phos-

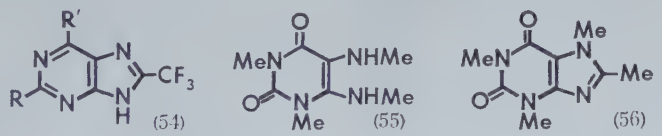


Reagents $HCONH_2/HCO_2H$ *and* $NaNO_2/Na_2S_2O_4$ 1, *at 130°C;* 2, *at 200°C;* 3, *at 200°C*

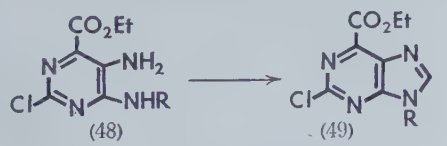
phoryl chloride, the method being noteworthy because of the advantages it offers. The main one is that the conversion of a chloro-4,5-diaminopyrimidine into a chloropurine may be made directly without hydrolysis of the chlorine atom occurring. Furthermore, the conditions



are extremely mild and in many cases the reaction proceeds at room temperature. Thus, the method has possible value for cyclizing pyrimidines containing labile groups. The



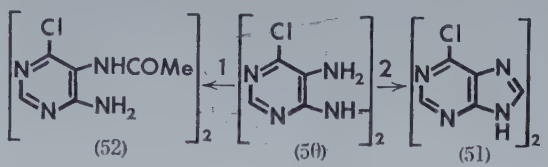
chloro pyrimidines (48) are readily converted into the purines (49)[63] and derivatives of xanthine, hypoxanthine and 6-methylpurine have also been obtained in this manner[63]. In



R = H, CH_2CO_2Et and CH_2CH_2Cl

Reagents $POCl_3/DMF$

one instance[64] cyclization of the bis-diamino-pyrimidine (50) to the chloropurine (51) was achieved when the existing method of forming a chloropurine directly, i.e. with an acetic anhydride-triethyl orthoformate mixture (see Section 3.2) gave only the 5-acetamidopyrimidine (52).



Reagents 1, $Ac_2O/(EtO)_3CH$; 2, $POCl_3/DMF$

The method can be regarded as an extension of the established Vilsmeier-Haack procedure[65] for introducing aldehyde groups into heterocyclic nuclei.

3.2 *Cyclizations involving anhydrides and* ortho *esters*

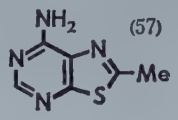
Acetic anhydride by itself is generally used for the direct conversion of 4,5-diaminopyrimidines into 8-methylpurines (53)[10,15,17,66,67].

Heating under reflux is sufficient to afford the purine, but by using milder conditions (<100°C) or mixtures with acetic acid the 5-acetamido intermediate[44] can be isolated. In

some cases acetylation of both amino groups occurs giving rise to 4,5-bisacetamido pyrimidines[16,68]. Trifluoroacetic anhydride alone or with trifluoroacetic acid has been used to prepare 8-trifluoromethyl purines[69] such as (54; R = R′ = H; R = R′ = NH_2; R = NH_2, R′ = CF_3). When 4,5-diamino-6-chloropyrimidine is treated with the anhydride alone hydrolysis of the chlorine atom occurs and 8-trifluoromethyl hypoxanthine (54; R = H, R′ = OH) is obtained. With a 4,5-bis-methylaminopyrimidine (55) cyclization results in the loss of methyl from the 4-methylamino group and 1,3,7,8-tetramethylxanthine (56) results[70].

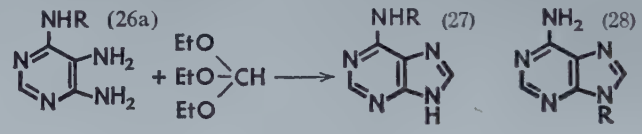
As has been noted both with formic acid and formamide cyclizations the presence of a 6-

mercapto group can lead to formation of a thiazolopyrimidine. This was also the case with 4,5-diamino-6-mercaptopyrimidine which gave 7-amino-2-methyl(5,4-*d*)thiazolopyrimidine (57) on heating with acetic anhydride[67].



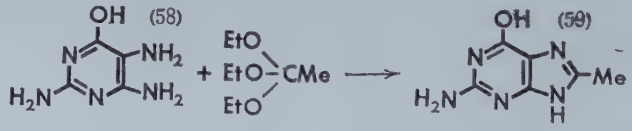
(57)

A more important role of anhydrides in purine synthesis is their use in conjunction with trialkyl orthoesters. The efficacy of this combination was discovered independently and simultaneously by two groups[71,72]. The anhydride-orthoester mixture is able to cyclize many types of 4,5-diaminopyrimidines in one step[27,73] and is sometimes successful when simpler methods fail. This was the case with 2,4,5-triamino-6-hydroxypyrimidine (58) which did not react with acetic anhydride alone but gave 8-methylguanine[67] (59) on the addition of triethyl orthoacetate to the reaction mixture. Its most outstanding advantage lies in its ability to produce 2- and 6-chloropurines directly from the chloro-4,5-diaminopyrimidines. These chloropurines are of great value because their chlorine atoms readily undergo displacement by nucleophilic reagents so that a variety of substituted purines can be prepared from them. Previously the only route to such chloropurines involved a tedious chlorination of the corresponding hydroxy compounds, a method not noted for giving good yields.



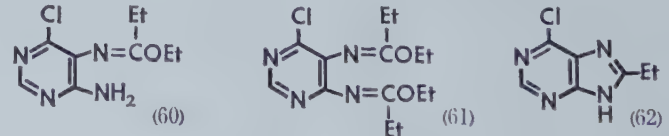
Reagent Ac_2O

One useful application of this reaction is to the ring closure of 4,5-diamino-6-furfurylaminopyrimidine (26; R = furfuryl) which gives the cell growth factor 'kinetin' (27; R = furfuryl)[75]. This mode of cyclization is again exemplified by the synthesis of the 6-hydroxyethylaminopurine (27; R = CH_2CH_2OH) from the 4-2′-hydroxyethylaminopyrimidine (26; R = CH_2CH_2OH). In contrast when the above



pyrimidines (26; R = CH_2CH_2OH)[76] and (26; R = furfuryl)[75] are subjected to a formamide cyclization the isomeric purines (28; R = CH_2CH_2OH) and (28; R = furfuryl) result.

The use of this mixed reagent has occasionally given a mixture of purines and *N*-acylated purines[72,74,75]. Because the absorption spectra of the latter are similar to those of the parent purines, acetylation is presumed to occur on either the 7 or 9 nitrogen atom[72]. In the above example of 'kinetin', however, the acetyl group is assigned to the extranuclear amino group[75]. In all cases the effects of acetylation can be nullified if alkali is used during the working up

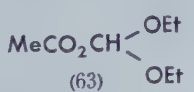


In this type of cyclization the anhydride no longer acts as an acylating agent and the 8-carbon atom with its substituent alkyl group

treatment, even cold dilute alkali is sufficient to cause hydrolysis.

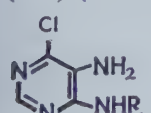
Purines having a methyl or ethyl group at the 8-position are obtained by replacing the triethyl orthoformate respectively by the orthoacetate ($(EtO)_3CMe$)[74,77] or orthopropionate ($(EtO)_3CEt$)[74]. Some difficulty has been encountered with triethyl orthopropionate[74] which is used in the absence of acetic anhydride. The initial reaction gives rise to a 5-(1-ethoxypropylideneamino)pyrimidine of the type (60), as well as small amounts of this bis derivative (61). Both are converted into the 8-ethylpurine (62) by heating them alone, or better still, with dimethyl sulphoxide, but neither method gives a good yield.

A more recent cyclizing agent is diethoxymethyl acetate (63)[78] which may be prepared from acetic anhydride and triethyl orthoformate. This ester has been used for many purine preparations[79,80], mainly chloropurines, for which it was found to be superior to using orthoformate-anhydride mixtures. A comparison of the two procedures[79] in the preparation of 2,6-dichloropurine (10; R = R′ = Cl) showed that diethoxymethyl acetate improved the yield by almost 50%. A mixture of triethyl orthoformate and diethoxymethyl acetate has also been tried but found to be less effective[81] than diethoxymethyl acetate alone.



(63)

Diethoxymethyl acetate has proved useful in the preparation of analogues of 6-chloropurine nucleosides in which cyclic alcohols take the place of the sugar moiety. Both the *cis* and *trans* forms of the cyclohexanol derivatives (65a) (*cis* form shown) are obtained in moder-



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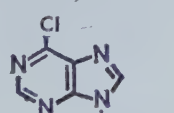
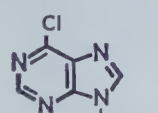


(65a)



(65b)

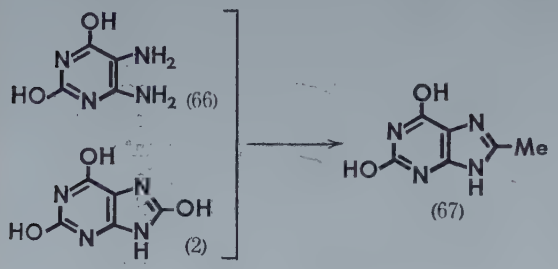
ate yields from the appropriate diaminopyrimidine (64; R = *cis* and *trans* cyclohexan-2-ol)[82] although some slight esterification of the *cis* alcohol is found. In contrast to this behaviour, large differences in reactivity are noted on cyclization of the *cis* and *trans* cyclopentanol derivatives (64; R = *cis* and *trans* cyclopentan-2-ol)[83]. Whereas the *cis* cyclopentanol (65b; as shown) is obtained in good yield using diethoxymethyl acetate the corresponding *trans* derivative under the same treatment suffers decomposition. It can be satisfactorily prepared by using triethyl orthoformate instead. It is of interest to note that if this reagent is used with the *cis* compound, in place of the diethoxymethyl acetate, no ring closure occurs. These differences in reactivity are presumed to be due to steric factors.

3.3 *Cyclizations with amides (other than formamide), ureas and their derivatives*

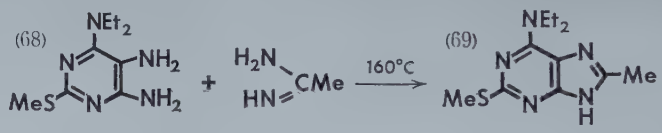
Syntheses of this type, in which initially both reactants are solids, only achieve cyclization if the reagents are fused. As this is, of necessity, a fairly drastic reaction, its use is limited to the preparation of purines containing nonlabile groups. The method, however, is of great utility not only because it allows the direct synthesis of 8-alkylpurines but, more particularly, in that it can yield purines containing groups at the 8-position capable of undergoing further reactions.

The 8-alkylpurines are prepared by heating the diaminopyrimidine with the amide, usually at the boiling point of the latter[30] for some hours. If the reaction time is reduced to minutes the intermediate 5-acylaminopyrimidine is obtained. In this way acetamide gives 8-methylpurines[18,30] and propionamide[30] the corresponding 8-ethyl derivatives whilst trifluoroacetamide (CF_3CONH_2) produces 8-trifluoromethylpurines[69]. A surprising feature is that the presence of a mercapto group at the 6-position of the diamine does not appear to complicate the reaction. When acetamide is fused with 4,5-diamino-6-mercapto pyrimidine only 6-mercapto-8-methyl purine is obtained[18]. The method has been extended so that 8-hydroxypurines themselves are converted into their 8-alkyl analogues by heating them with the appropriate amide[84]. For this type of reaction, in which a hydroxyl group is apparently replaced by an alkyl group, ring opening of the imidazole ring must precede subsequent cyclization by the amide. For example, uric acid (2) with acetamide gives 8-methylxanthine (67) which is also obtained by acetamide treatment of 4,5-diamino-2,6-dihydroxypyrimidine (66).

Some use has also been made of the amidines for direct cyclization, thus acetamidine in the

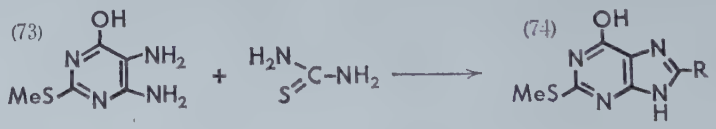


form of its hydrochloride, when fused with the triaminopyrimidine (68) gives the 8-methylpurine (69)[41]. Urea and thiourea have been



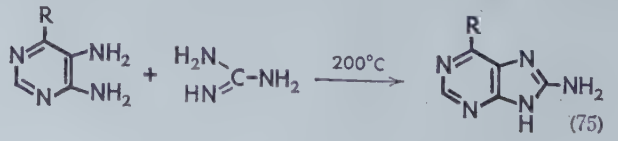
extensively used in the Traube synthesis and some of the earliest purine preparations were made with these two reagents[85].

Where a choice is available, urea is to be preferred from a practical standpoint. The fusion temperature required is generally lower than that needed for the thio- derivative (~180°C compared with ~200°C) and a cleaner product is usually obtained. The purine

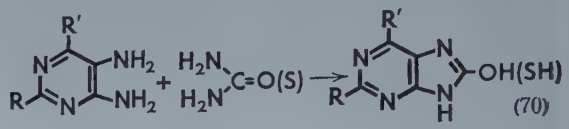


is recovered as an insoluble residue when the melt is dissolved in water.

The 8-hydroxypurines are readily chlorinated by phosphoryl chloride-diethylaniline



mixtures (see ref. 89) whilst methylation of the 8-mercapto analogues with methyl sulphate affords the 8-methylmercaptopurines. In this way a choice of active groups is available for further replacement.



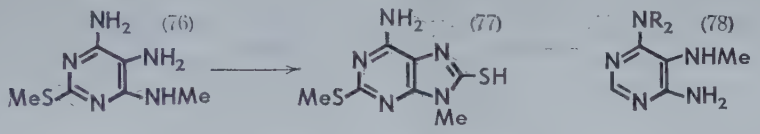
The purines made by this procedure range from simple 8-hydroxy- and 8-mercaptopurines such as (70)[15] to the more complex polyhydroxy- and polymercapto- derivatives[86] and *N*(1)- and *N*(3)-methylated purines[86–88]. Both 7- and 9-alkyl substituted 8-hydroxypurines can be prepared from the appropriate 4- or 5-substituted aminopyrimidines[32,66]. That the nitrogen atoms attached to the pyrimidine are retained is shown when *NN'*-dimethylurea is fused with 4,5-diaminopyrimidine (71) and only the 8-hydroxypurine (72) is obtained[90].

One instance where a thiourea fusion gives rise to an anomalous cyclization is observed with the pyrimidine (73). In place of the expected 6-hydroxy-8-mercapto-2-methylmercaptopurine (74; R = SH) the 8-amino derivative (74; R = NH_2) is obtained[91].

When an unambiguous synthesis of an 8-aminopurine is required, guanidine, in the form of its salt, is used. Both the 8-amino-6-hydroxy- (75; R = OH) and 8-amino-6-mercaptopurines (75; R = SH) are obtained by this route[89] and for the latter compound no interaction of the guanidine with the mercapto group has ever been reported.

3.4 *Cyclizations with simple derivatives of carbon disulphide and carbon dioxide*

Many 8-mercaptopurines can be prepared by heating the diaminopyrimidine in pyridine with carbon disulphide[13,19,27]. The procedure was first used in the preparation of 8-mercaptoxanthine (8-mercaptouric acid)[92]. When compared with the alternative method of fusion with thiourea, the advantage conferred by the lower reaction temperature used is readily appreciated. The product is isolated simply by removal of the solvent.



Reagent CS_2 and pyridine

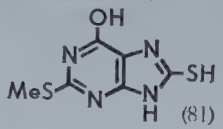
When both a primary and secondary amino group are present as in the pyrimidine (76) cyclization occurs between the 5-amino and the secondary amino group and the product is the 9-substituted purine (77)[93]. Attempts to ring close some 5-methylaminopyrimidines have not given 8-mercapto-7-methylpurines. Both (78; R = H)[94] and (78; R = Me)[95] failed to cyclize and only starting material was recovered. The same workers[95] also found that the latter compound did not undergo reaction with formic acid either.

Caution should be observed with *N*-methylated pyrimidines as one example is reported where an attempted cyclization led to a migration of the methyl group. Thus from carbon disulphide treatment of the *N*(3)-methyl pyrimidine (79) in pyridine was isolated 2-hydroxy-8-mercapto-9-methylpurine (80)[87]. Although

methyl group migrations from nuclear to extranuclear nitrogen atoms are known to occur

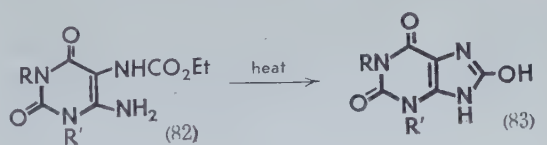
with purines the above example does not fit the mechanism postulated[96]. However, if isomerisation is assumed to take place prior to cyclization as has been observed with other methyl substituted pyrimidines[97], then the mechanism of the reaction is explicable.

Thiophosgene is also used[66] to prepare 8-mercaptopurines of the type (81) from the corresponding diamine (73).



Carbon dioxide, itself, has been used (see below) but its derivatives, phosgene and, to a lesser extent, ethyl chloroformate ($ClCO_2Et$), are mainly employed. With phosgene the reaction is generally carried out by bubbling the gas through an alkaline solution of the pyrimidine. In this way a number of 8-hydroxypurines, for example (70; R = H, R′ = NH_2; R = H, R′ = OH; R = OH and R′ = NH_2) are obtained directly[99].

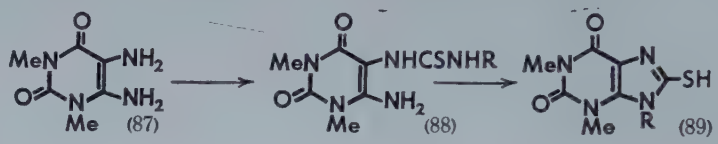
Ethyl chloroformate also reacts with diaminopyrimidines in alkaline solution but the process is a two-stage one and the intermediate urethane (82) so formed must be isolated as the sodium salt. This is then converted by heat to the 8-hydroxypurine (83). Traube[8]



used this route to synthesize uric acid (70; R = R′ = OH) as well as its *N*(1)-methyl (83; R = Me, R′ = H), *N*(3)-methyl (83; R = H, R′ = Me) and *N*(1)*N*(3)-dimethyl (83; R = R′ = Me) homologues. In a like manner the *N*(3)-ethyl uric acid has also been prepared[100].

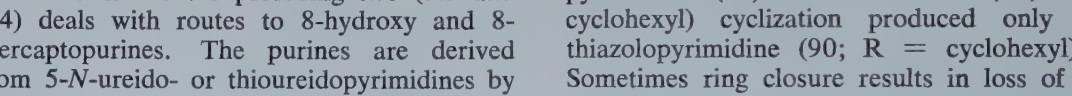
It is noteworthy that phosgene does not appear to interact directly with 4,5-diaminouracil (66) but the reaction can be accomplished by converting the diamine to the tri-

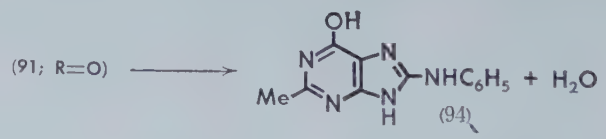
methylsilyl derivative (85) using 1,1,1,3,3,3-hexamethyldisilazane (84)[98]. After phosgene treatment in toluene the resulting purine still carries trimethylsilyl groups at the 2, 6, 7 and 9-positions but these are removed by hydrolysis with aqueous ethanol to give uric acid (2). This 'activated' form of the diaminopyrimidine (85) will also react directly with carbon dioxide to give uric acid. Sealed tube conditions are employed and an excess of the disilazane is used as solvent.

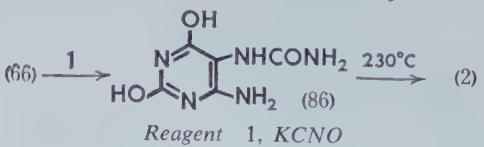


3.5 *Cyclizations with cyanates and isothiocyanates*

This section like the preceding two (3.3 and 3.4) deals with routes to 8-hydroxy and 8-mercaptopurines. The purines are derived from 5-*N*-ureido- or thioureidopyrimidines by a cyclization in which the elements of ammonia are withdrawn. Alkyl or aryl cyanates, isothiocyanates or dithiocarbamates are used to prepare the ureido derivatives which on conversion give the corresponding 9-alkyl or -aryl purines. Cyanic acid can be used for the preparation of 9-unsubstituted purines. Uric acid was obtained by Traube[10] in this way using potassium cyanate to form the ureido derivative (86) which was converted to uric acid (2) by heating it alone or with dilute hydrochloric acid. The use of phenyl cyanate and phenyl isothiocyanate gave 9-phenyl uric acid and 9-phenyl-8-thiouric acid respectively[10]. Later workers[101] made use of this procedure to prepare purines of the type (89; R = Me, Et, Pr, Bu, $CH_2C_6H_5$ and $CH_2C_6H_{11}$). It should, however, be pointed out that this route to 8-mercaptopurines is not as reliable as either the carbon disulphide or thiourea routes. The main disadvantage is that a variety of products may result from the cyclization step which can proceed by more than one pathway. Thus, a large proportion of the 8-mercaptopurines obtained in the above preparation were found associated with the isomeric (5,4-*d*)thiazolopyrimidines (90) and in one case (88; R = cyclohexyl) cyclization produced only the thiazolopyrimidine (90; R = cyclohexyl)[101]. Sometimes ring closure results in loss of the ω-alkyl group giving rise to a purine unsubstituted at the 9-position. This is found with the thioureidopyrimidine (91; R = S) which on heating in pyridine loses aniline giving the purine (92)[102]. A similar result is obtained with the ethyl thioureidopyrimidine (93)[101] which evolves ethylamine and gives *N*(1)*N*(3)-dimethyl-8-mercaptoxanthine (89; R = H).



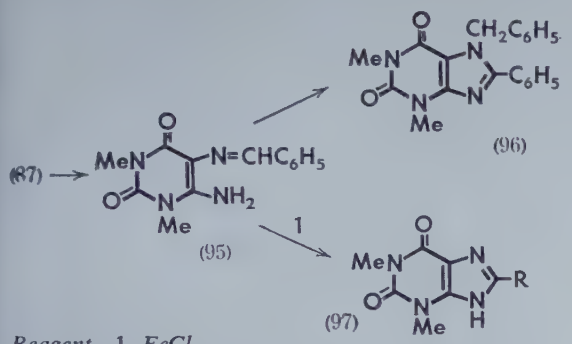




Loss of ethylamine from a similar thioureido derivative is reported by other workers[94] but no conditions are specified. It is also possible to effect cyclization of a ureidopyrimidine via the carbonyl group with the removal of the elements of water rather than ammonia. In this way an 8-aminopurine (94) results from cyclodehydration of the ureidopyrimidine (91; R = O) by heating it in toluene in the presence of phosphorus trichloride[102].

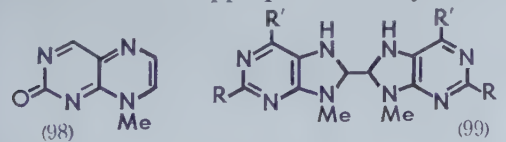
3.6 *Miscellaneous cyclizing reagents*

Under this heading are grouped a few unusual reagents which do not fall conveniently into the preceding sections. Generally speaking they are of limited application or give only moderate yields. Several workers have investigated the possibilities of using aldehydes, both aliphatic and aromatic, as cyclizing agents. Traube[103] condensed one molecular equivalent of benzaldehyde with the diaminopyrimidine (87) obtaining the Schiff's base (95). This, on the



Reagent 1, $FeCl_3$

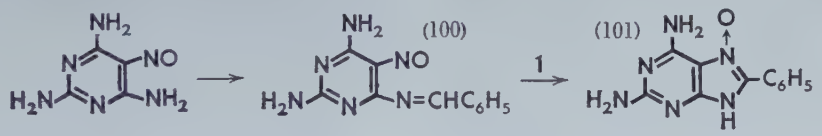
addition of a further mole of benzaldehyde, cyclized to give 7-benzyl-1,3-dimethyl-8-phenylxanthine (96). If, however, an oxidizing agent is present then (95) is readily converted into a 1,3-dimethyl-9-phenylxanthine (97; R = C_6H_5). Other alkyl and aryl purines (97; R = H, Bu, and *o*-C_6H_4OH) are similarly prepared from the appropriate aldehyde using ferric chloride as the oxidizing agent. One unexpected purine preparation has arisen from an attempted Isay type pteridine synthesis[104]. When glyoxal is added to 5-amino-2-hydroxy-4-methylaminopyridine the product is not the pteridine (98) but the bis-dihydropurinyl (99).



When other diketones, *e.g.* diacetyl, benzil, are employed the normal reaction ensues and the corresponding 6,7-disubstituted pteridines are produced. These results are explained by assuming that the small size of the glyoxal molecule allows an initial interaction with the 4-methylamino group which is followed by cyclization to the five-membered ring. With the larger diketone molecules, steric factors permit only a normal type of Schiff's base condensation with the 5-amino group and a subsequent ring closure to pteridines[105]. Analogous bis-dihydropurinyls are obtained on treatment of other 4,5-diamino[23] and 5-amino-4-methylaminopyrimidines[104] with glyoxal.

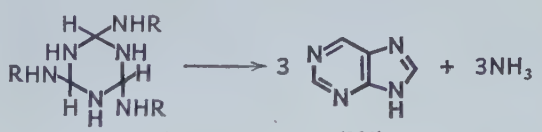
Aldehydes also react with 4-amino-5-nitrosopyrimidines; two molecular equivalents of the former with nitroso derivatives give purines. The method has been modified[106] by using benzaldehyde anil ($C_6H_5N{=}CHC_6H_5$) to prepare purine-*N*(7)-oxides of the type (101).

The observation that *sym*-triazine is degraded by primary amines into *NN'*-disubstituted formamidines has been ingeniously utilized for purine synthesis[107]. By replacing the primary amine with a 4,5-diaminopyrimidine an addi-

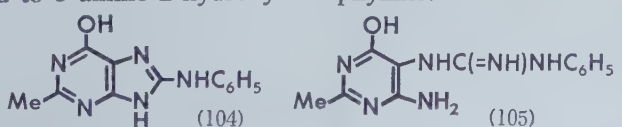


Reagent 1, *acetic acid*

tion complex of the type (102; R = 4-amino-5-pyrimidinyl) is first formed, presumably through the 5-amino group, which then breaks down into three moles each of purine (103) and ammonia.



This technique has also been successfully applied to the formation of xanthine and theophylline.

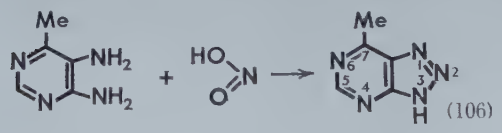


Cyanogen bromide with 4,5-diaminopyrimidine gives 8-aminopurine in poor yield[15]. An

8-substituted aminopurine (104) was obtained by the action of phenyl cyanamide (C_6H_5NH-CN) on a diamine in boiling butanol[102]. That the reaction does not proceed via the intermediate guanidino pyrimidine (105) seems to be shown by the failure to cyclize this derivative to the purine (104).

3.7 *Purine analogues by the Traube synthesis*

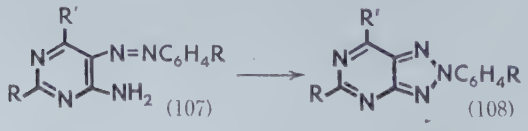
In view of the number of purine analogues currently being synthesized as potential 'antipurines' it is pertinent that some mention be made of the application of the Traube synthesis to their preparation. Purines in which the methine group at the 8-position is replaced by a heteroatom are obtained by this route. Possibly the earliest of these analogues were the 8-azapurines (v-triazolo(5,4-*d*)pyrimidines) of which the 6-methyl derivative (106) appears to be the first reported[85]. Their preparation consists of treating the diaminopyrimidine with nitrous acid at just below room temperature[21,33,39,73,75] but amyl nitrite at higher temperatures has been used also[108]. Under these



(106)

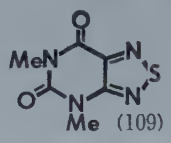
mild conditions potentially labile groups may be present thus both chlorine-[108] and sugar-containing triazoles[108a] can be obtained. When 4,5,6-triaminopyrimidines are used, in which either the 4- or 6-amino group is secondary, only one isomer results, this being substituted at the 3-position[108a,109].

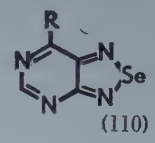
Copper sulphate oxidation of 5-phenylazopyrimidines of the type (107) provides a route to 2-substituted triazolopyrimidines (108)[110-112], the method being analogous to the ferric chloride oxidation of Schiff's bases to 8-substituted purines, described in the previous



Reagents $CuSO_4$ *and pyridine*

section. The introduction of sulphur and to a lesser extent selenium has also been investigated. The action of thionyl chloride on the diamine (87) gives the 8-thiapurine (109) in



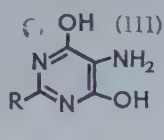


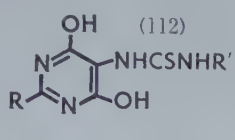
good yield[113]. Later this method was employed to prepare other derivatives of this type[114]. As well as thionyl chloride, both sulphur dichloride and sulphur chloride have been tried[115] but with less success. Although not strictly a Traube type synthesis the fusion of 4-amino-5-nitrosopyrimidines with thiourea provides a novel route to 8-thiapurines[114] and confirmation of the structures of the products is obtained from their alternative synthesis from the appropriate diamine and thionyl chloride. The importance of sulphur compounds in living systems has led to studies of the selenium derivatives as well. This being the case with the 8-thiapurines, a number of 8-selenapurines of the type (110; R = OH, NH_2 and morpholyl) have been prepared for biological testing by the action of selenous acid on the diamine in the cold[116]. This preparation is not new, however, as the selenium analogue of (109) has been known for many years[117].

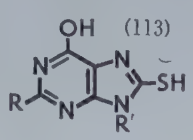
4 SYNTHESES FROM 5-AMINO-4-HYDROXY- AND 4,5-DIHYDROXY-PYRIMIDINES

Under this heading are grouped the remaining preparations of purines from pyrimidines. Fischer's route[5] to uric acid (see Section 2) is one of the first examples of the most widely used alternative to the Traube synthesis. The starting material, 5-amino-2,4,6-trihydroxypyrimidine (uramil, 111; R = OH), is condensed with alkyl or aryl cyanates or isothiocyanates and the resulting 5-*N*-ureido- or -thioureidopyrimidines (112), the 'pseudo uric acids' and 'pseudo thiouric acids' of Fischer, are cyclized to 9-substituted-8-mercaptopurines (113) on treatment with refluxing mineral acid. The isothiocyanates are most commonly employed as their use leads to the more versatile 8-mercaptopurines. Thus various derivatives ranging from the simple 9-methyl and 9-ethyl purines[118] (113; R = OH, R′ = Me and Et) and their *N*-methylated analogues[118] to more complex 9-aryl purines[119] have been

obtained in this way. At one time it was thought that only uramil would undergo the above reaction to produce purine derivatives. However, some success has been met with using 2,5-diamino-4,6-dihydroxypyrimidine (111; R = NH_2) to prepare 9-substituted-8-mercaptoguanine derivatives[119] of the type (113; R = NH_2). When alkyl isothiocyanates are em-

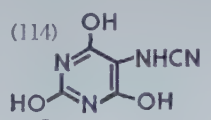






ployed the thioureido derivatives (112; R = NH_2, R′ = Me, Et and *i*-Bu) are readily cyclized by acid treatment to the guanines (113; R = NH_2, R′ = Me, Et and *i*-Bu). On the other hand, although aryl isothiocyanates do yield the corresponding thioureidopyrimidines (112; R = NH_2, R′ = aryl) attempts at ring closure, even under mild acid conditions result only in degradation products being obtained.

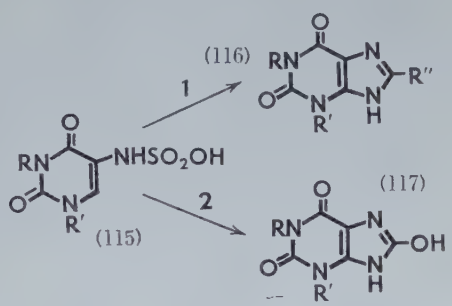
One approach to thioureidopyrimidines, now only of historical interest, was devised by Fischer[120] who treated the 5-cyanamido-derivative (114) of uramil with ammonium sulphide



and obtained the thioureidopyrimidine (112; R = OH, R′ = H). On acid treatment this was converted into the purine (113; R = OH, R′ = H). The method is limited in application to the preparation of 8-mercaptoxanthines.

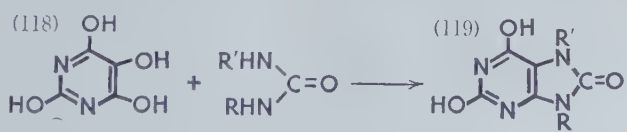
Of more recent date is a novel route which utilizes 5-sulphamino uracils (115; R = R′ = H). These undergo cyclization on heating with amides giving the appropriate 8-alkylxanthines (116; R = R′ = H, R″ = alkyl) or uric acid (117; R = R′ = H) on fusion with urea[121].

The reaction has been further studied using *N*-methylated sulphamino pyrimidines[58]. Whilst on the one hand the *N*(3)-methyl derivative (115; R = H, R′ = Me) does undergo the above reaction with formamide, acetamide and urea giving the respective *N*(3)-methyl homologues of xanthine (116; R = R″ = H, R′ = Me), 8-methylxanthine (116; R = H, R′ = R″ = Me) and uric acid (117; R = H, R′ = Me) the corresponding *N*(1)-methylsulphamino uracil (115; R = Me, R′ = H) with the above amides gives only the acylated 5-amino-*N*(1)-methyluracils.



Reagents 1, $R''CONH_2$; 2, $CO(NH_2)_2$

Purine synthesis starting from 4,5-dihydroxypyrimidines (118) are little used in the present day. In essence the 4,5-dihydroxypyrimidine is fused with a urea or substituted urea. When *N*-methylurea is condensed with 2,4,5,6-tetrahydroxypyrimidine (118) a 7-methyl uric acid (119; R = H, R′ = Me) results and the 7,9-dimethyl analogue (119; R = R′ = Me) is obtained from *NN*′-dimethyl urea[122]. These last preparations serve to demonstrate the ease of scission of the C–O bonds at the 4- and 5-



positions of the pyrimidine ring. In contrast the C–N bonds in the corresponding 4,5-diamino pyrimidines are retained intact under the conditions of the urea fusion (see Section 3.3).

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Naturally Occurring Aliphatic Hydroxyacids

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1 INTRODUCTION

Recent years have witnessed a great increase in the knowledge of the chemistry of lipids, which are loosely defined as those products of living organisms which are soluble in organic solvents and which, therefore, are principally aliphatic or alicyclic in composition. This progress has been stimulated by the needs of biochemists and largely made possible by the development of new techniques. The interest with which organic chemists have watched this development is shown by the intense effort which they have applied when lipids of novel structure or activity have been discovered. Such interest has been only sporadic and has resulted in haphazard development in this field with the result that new components and sometimes new classes of compounds are still being discovered in common fatty materials.

Until comparatively recently the fatty hydroxyacids which were occasionally isolated from natural sources were regarded as curiosities. They have continued to be encountered with increasing frequency and certain of them, the α-hydroxyacids, are now suspected to be general cell constituents. Long-chain hydroxyacids often serve as structural materials in plants and animals and a logical connection between structure and function is sometimes apparent. Certain hydroxyacids, or derivatives of hydroxyacids, have been thoroughly investigated because they have been found to exhibit specific biological activities but as a class the hydroxyacids appear to have been neglected and the composition of mixtures of hydroxyacids present in common biological materials often remains largely unknown.

In this review the naturally occurring hydroxyacids of low molecular weight, such as lactic acid, which already have an extensive biochemical literature of their own, have not been considered. Those of somewhat more complex structure, such as occur in esters in the pyrrolizidine group of alkaloids[1], have likewise been regarded as outside the scope of this review.

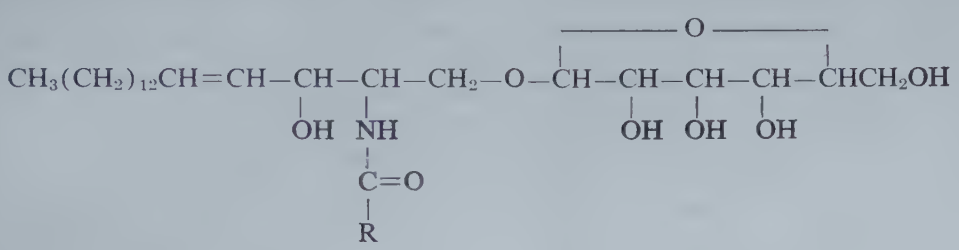
2 NOMENCLATURE

Since the general adoption of the Geneva system of nomenclature and the periodical recommendations of the IUPAC commissions[2] there has been a welcome abatement in the use of trivial names for aliphatic materials, the confusing proliferation of which occasioned an eloquent protest by Chibnall and Piper[3].

The advantages of the present system of numbering the positions of substitution of fatty

acids, in which the carboxyl carbon atom is taken as position 1, require no comment. Use is still commonly made of the Greek letters α, β, γ, δ, and ω to denote respectively the positions 2, 3, 4, 5, and that furthest from the carboxyl group, reflecting the convenience of this system, particularly in speech and for the designation of classes of compounds. Similar justification can be made for the retention of certain trivial names of aliphatic radicals, such as palmitic, stearic and oleic, and those of less than five carbon atoms, and this course is sanctioned in recent IUPAC recommendations.

tively known as cerebrosides, have since been shown to be compounds of sphingosine, galactose (or sometimes glucose), and fatty acids. They have been found to occur in most organs from a wide range of animals and similar compounds also occur in plants and microorganisms[6]. In phrenosin the acids have a hydroxyl group in the α-position, and both phrenosin and cerasin contain unsaturated acids. Investigations leading to the structures shown in Figure 1 are reviewed elsewhere[7–10] and consideration of them here will be limited to the composition of the fatty acid moiety.



Cerasin, R = $CH_3(CH_2)_nCH_2-$ or $CH_3(CH_2)_xCH=CH(CH_2)_yCH_2-$
Phrenosin, R = $CH_3(CH_2)_nCH(OH)-$ or $CH_3(CH_2)_xCH=CH(CH_2)_yCH(OH)-$

Figure 1

Frequent use is also made of the prefix *'iso'* to denote the homologous series of compounds derived from *iso*-butyric acid. The term *'anteiso'* was coined by Weitkamp[4] for the homologous series of acids carrying a methyl substituent on the antepenultimate carbon atom away from the carboxyl group (1 and 2). These two terms have gained wider usage now that the corresponding series of fatty acids are recognized as normal constituents of animal fats.

$$(CH_3)_2CH(CH_2)_nCO_2H \qquad (1)$$
iso series

$$CH_3CH_2CH(CH_3)(CH_2)_nCO_2H \qquad (2)$$
anteiso series

3 HYDROXYACIDS FROM ANIMALS

3.1 *Cerebrosides*

Cerasin and phrenosin, two sparingly soluble fractions of brain lipids, were isolated and named by Thudichum[5] in 1874. These, collec-

Until recently, phrenosin was separated from the lipids of nervous tissue and other organs by crystallization. This was followed by hydrolysis and crystallization of the fatty acids resulting in material which was believed by Levene[11] to be 2-hydroxypentacosanoic acid, and by Klenk[12] to be 2-hydroxytetracosanoic acid. Chibnall, Piper, and Williams[13, 14] concluded from melting point and X-ray diffraction data that the material was a mixture of 2-hydroxy-*n*-alkanoic acids with 22, 24, and 26 carbon atoms. The methods used in these studies were such that minor and more soluble constituents of the original mixture might easily have been overlooked. That at least one further acid is present in beef spinal chord phrenosin was indicated when Skipski, Arfin, and Rapport[15] detected 2-hydroxystearic acid by paper chromatography.

Kishimoto and Radin[16] used adsorption chromatography to achieve an almost quantitative separation of the cerebrosides from rat

brain and of the derived methyl esters of the hydroxyacids. These were separated into saturated and unsaturated fractions by means of the mercuric acetate adduct of the unsaturated compounds which formed about 10% of the esters. Both saturated and unsaturated fractions were then examined by gas chromatography and found to contain normal α-hydroxyacids with 20, 21, 22, 23, and 24 carbon atoms together with higher and lower molecular weight compounds which appeared from their retention times to be derivatives of branched chain α-hydroxyacids. Downing[17] investigated the α-hydroxyacids of sheep brain by separation of the derived α,β-diols, as their isopropylidene derivatives, from the alcohols obtained on reduction of the methyl esters of the acids liberated by hydrolysis of the whole of the brain lipids. Gas chromatographic analysis showed that all chain lengths from 14 to 26 carbon atoms were represented and that branched-chain compounds were absent.

Klenk[18] found evidence for the presence in phrenosin of 2-hydroxytetracos-15-enoic acid. His report of the occurrence of both the Δ^{15} and Δ^{17} isomers[19] must remain suspect because he was probably working with a mixture of compounds with different chain lengths.

The α-hydroxyacids of phrenosin are optically-active; comparison of the optical rotations of some derivatives with corresponding derivatives of authentic αD-hydroxyacids led to the conclusion that the brain hydroxyacids also have the D-configuration[20]. Horn and Pretorius[21] established this point by synthesis of a series of hydroxyacids by anodic coupling of normal fatty acids with a half-ester of L(−)-malic acid (Figure 2). It was unnecessary to separate the two half esters since acids

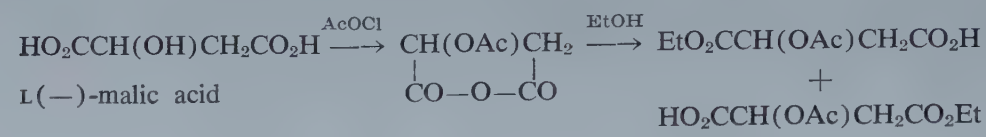


Figure 2

with an α-substituent do not undergo anodic coupling. The αL-hydroxyacids thus formed were the enantiomorphs of the hydroxyacids from brain phrenosin and from wool wax (Figure 3).

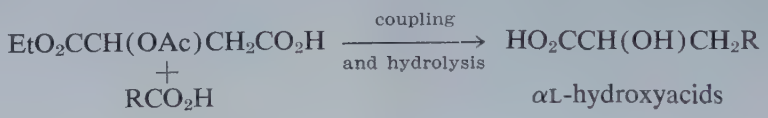


Figure 3

3.2 *Wool wax*

As a result of the commercial importance of wool wax and the vast quantities of this material which are available for increased industrial exploitation this material is the most thoroughly examined natural wax. The presence of hydroxyacids was first suspected by Lewkowitsh[22, 23] in 1892, and established by Kuwata[24], who isolated and identified α-hydroxypalmitic acid. Weitkamp[4] developed low-pressure distillation methods which enabled him to isolate two α-hydroxyacids, the C_{14} and C_{16} normal compounds, while Horn and co-workers[25] were similarly able to isolate the methyl esters of the normal C_{12}, C_{14}, and C_{16} α-hydroxyacids from a concentrate obtained by counter-current distribution of the methyl esters of wool wax acids. Higher homologues were too thermolabile to be distilled but the presence of 2-hydroxystearic and 2-hydroxy-16-methyl-heptadecanoic acids was shown by distillation of the acetates of the α, β-diols obtained on lithium aluminium hydride reduction of the mixture of α-hydroxyacids methyl esters. Subsequently, Downing, Kranz and Murray[26] investigated the composition of wool wax by gas chromatographic methods and identified twenty-five α-hydroxy-

acids, belonging to the normal, *iso*, and *anteiso* series, with chain-lengths from 13 to 25 carbon atoms. A feature of the α-hydroxyacid mixture was the preponderance of the *n*-C_{16} and *iso*-C_{18} acids, which amounted to 40% and 24% respectively, whereas no other constituent formed more than 5% of the mixture. The α-hydroxyacids represent about 30% of the total acids of wool wax, which in turn constitute nearly half the wax.

Higher molecular weight hydroxyacids, which have long been known to occur in wool wax, were separated from the hydrolysed wax by Horn and Pretorius[27] as the sparingly-soluble potassium salts. Low pressure distillation of the cyclic lactones, prepared by the method of Stoll and Rouvé[28], resulted in the isolation of 30-hydroxytriacontanoic and 32-hydroxy-dotriacontanoic acids. Downing, Kranz, and Murray[26] subsequently showed that this group of acids, representing 5% of the acids of wool wax, also contains branched-chain hydroxyacids. At least eleven acids are present, including the 26-, 28-, 30-, 32-, and 34-carbon normal acids, the 30- and 32-carbon *iso*-acids, and the 27-, 29-, 31-, and 33-carbon *anteiso* compounds. The position which the hydroxyl group occupies in each of the new acids remains to be determined. Even if it is primary, as in the two normal members isolated by Horn and Pretorius, the *anteiso* compounds could have either a methyl or ethyl side-chain.

3.3 *Beeswax*

The presence of hydroxyacids in beeswax was discovered by Toyama and Hirai[29] who were able to isolate and identify 14-hydroxypalmitic acid. A gas chromatographic examination[30] has shown that this is the major constituent of a mixture of hydroxyacids having chain lengths from 12 to 32 carbon atoms including the odd carbon numbered members. The mixture of hydroxyacids, amounting to 16% of the wax, was separated from the hydrolysis products of the wax and reduced to the corresponding mixture of hydrocarbons in order to provide a sufficiently stable material for gas chromatographic analysis, and thus no indication was obtained of the position of the hydroxyl group in any of these newly-demonstrated constituents.

3.4 *Bee mandibular glands*

Considerable interest was aroused by a report of the occurrence of 10-hydroxydec-2-enoic acid from royal jelly[31], the food of larval bees destined to become queens. The acid forms 15% of the jelly and 70% of the ether-soluble fraction[32] and has been found to occur also in the food of worker bee larvae. It originates in the mandibular glands of the foraging worker bees, whence it is added to the food of the young[33, 34], and has been shown to have weak antibiotic activity[32].

The constitution of the acid was elucidated by its reduction to 10-hydroxydecanoic acid and its oxidation to give *n*-octanedioic acid and oxalic acid[32]. The configuration of the double bond could not be determined by infrared spectroscopy, and it was eventually shown to be *trans* by nuclear magnetic resonance spectroscopy[34]. It has subsequently been shown that royal jelly contains at least ten saturated and unsaturated monohydroxyacids, in addition to dihydroxy acids containing a β-hydroxyl group, and two dicarboxylic acids[35]. The material which actually regulates the rearing of queen bees by the workers, the queen substance, has since been shown to be the related compound 9-oxodec-2-enoic acid[36]. It is secreted by the queen bee and ingested by the workers during their cleansing operations and this prevents them from rearing further queens and from developing ovaries themselves.

3.5 *Human faecal lipids*

James, Webb and Kellock[37] recently examined the faecal lipids of normal human subjects by gas chromatographic methods and demonstrated the presence of two groups of unusual fatty acids which did not occur in the diet. One of these groups consisted of positional and configurational isomers of oleic acid, while the other contained principally 10-hydroxystearic acid, with minor amounts of isomers in which the hydroxyl group occupied the 6-, 7-, 8-, and 9-positions. It was suggested that these hydroxyacids may be intermediates in the synthesis of the unusual unsaturated acids which microflora produce in the intestinal tract.

4 HYDROXYACIDS FROM PLANTS

Although fatty hydroxyacids are not generally regarded as common constituents of plants they have been isolated in wide variety from a range of plant materials and some may occur in all plants. Occasionally there is an obvious correlation between the structure of a hydroxyacid and its function in the plant.

4.1 *Bark, cuticle, and roots*

Suberin, the non-extractable saponifiable material which constitutes up to 40% of the weight of cork has been the subject of continuous study for a remarkable length of time. It is now known to contain several polyfunctional long-chain hydroxyacids which in the original material are inter-esterified to form a cross-linked insoluble polyester admirably suited to its function as a tough outer coating of trees.

Phellonic acid was originally isolated from suberin by Kugler[38] in 1884. Several structures were successively assigned to it by a number of workers[39,40] before it was shown by Jensen in 1950 to be 22-hydroxydecosanoic acid[41, 42], this structure having been since adequately confirmed[43, 44]. In addition suberin contains 9,10-dihydroxyoctadecanedioic acid[45] (phloionic acid), 9,10,18-trihydroxyoctadecanoic acid[45] (phloionolic acid), and eicosanedioic acid[46] (phellogenic acid). The corks from *Quercus suber* and *Betula verrucosa* are similar in their content of these acids[41, 47]. The corresponding material from the bark of *Pseudotsuga taxifolia* (Douglas fir) is claimed to contain 11-hydroxylauric acid, a hydroxypalmitic acid and a hydroxyhexadecenoic acid[48].

The cutin of plants is an insoluble elastic material which can be obtained apparently unchanged after removal of carbohydrates, proteins and soluble lipids. It is similar to suberin in composition and it also has been long known to contain hydroxyacids. Matic[49] has isolated from the cutin of *Agave americana* 10,16-dihydroxyhexadecanoic acid, 10,18-dihydroxyoctadecanoic acid and 9,10,18-trihydroxyoctadecanoic acid. The presence of 18-hydroxyoctadec-*cis*-9-enoic acid was shown by its oxidation with performic acid to give threo-9,10,18-trihydroxyoctadecanoic acid, which was shown to be identical with the naturally occurring trihydroxyacid from cutin and with phloionolic acid from suberin. The hydroxyacids were present in cutin as a cross-linked polyester soluble only after saponification. Similar compounds have been isolated from olive leaves[50]. The cutin from apple skin, isolated by digestion of other constituents of the skin by snail-gut enzymes, also appears to be similar[51].

Many of the hydroxyacids which have been isolated from plants have been discovered as a result of investigations of materials used in pharmacy such as the purgatives obtained from the roots of Ipomoea species and of aromatic oils like those obtained from angelica root oil and from the bark oil of *Cryptocarya massoia*. Such materials have principally been found in the roots and bark of plants, where the hydroxyacids are often present as lactones. These sources are listed in the Table.

4.2 *Leaf waxes*

The leaves of most plants are coated with a certain amount of waxy material which is sometimes present in sufficient quantities to be of commercial importance as with the carnauba palm. Murray and Schoenfeld[52] have found the saponifiable fraction of carnauba wax to contain a large proportion of ω-hydroxyacids and low pressure distillation of the acetylated methyl esters of these resulted in the isolation of the derivatives of ω-hydroxyacids having an even number of carbon atoms from 18 to 30. In a reinvestigation by gas chromatographic methods[53], small amounts of the odd-carbon numbered hydroxyacids from 21 to 31 were detected and the even-carbon numbered acids shown to extend from 16 to 34 carbon atoms, all of these being straight-chain compounds. Here again the hydroxyacids are believed to occur as polymers in the wax but since only difunctional compounds are present there can be no crosslinking of polymer chains and the material is soluble in hot organic solvents.

The leaf wax of many conifers is also a rich source of ω-hydroxyacids, the characteristic acid being 16-hydroxyhexadecanoic acid (juniperic acid), sometimes accompanied by 12-hydroxydodecanoic acid (sabinic acid), both of which were isolated in the course of the pioneering work of Bougault[54, 55]. These ω-hydroxyacids commonly form 80% of the conifer waxes in which they occur[56–59]. Higher

Table Sources of naturally occurring hydroxyacids

Source	Composition	References
ANIMALS		
Sheep (wool wax)	$CH_3(CH_2)_nCH(OH)CO_2H$ where n = 9,10,11, ... 21	22-26
	$(CH_3)_2CH(CH_2)_nCH(OH)CO_2H$ where n = 9,11,13, ... 19	
	$CH_3CH_2CH(CH_3)(CH_2)_nCH(OH)CO_2H$ where n = 7,9,11, ... 19	
	$HO(CH_2)_nCO_2H$ where n = 25,27,29,31,33	
	$HOCH_2CH(CH_3)(CH_2)_nCO_2H$ where n = 26,28 ?	
	$HOCH_2CH_2CH(CH_3)(CH_2)_nCO_2H$ where n = 22,24,26,28 ?	
Bees (beeswax)	$CH_3CH_2CH(OH)(CH_2)_nCO_2H$ where n = 12, and probably 8,9,10, ... 28	29, 30
Bees (mandibular glands)	$HO(CH_2)_8CH\overset{t}{=}CHCO_2H$	31-34
Animal cerebrosides	$CH_3(CH_2)_nCH(OH)CO_2H$ where n = 11,12,13, ... 23	12-17
	$CH_3(CH_2)_nCH=CH(CH_2)_mCH(OH)CO_2H$ where n = 5, m = 14; n = 7, m = 12; and $n + m$ = 9,10,11, ... 23	
Man (faecal lipids)	$CH_3(CH_2)_7CH(OH)(CH_2)_8CO_2H$	37
PLANTS		
Agave americana (cutin)	$HO(CH_2)_{17}CO_2H$	49
	$HO(CH_2)_6CH(OH)(CH_2)_8CO_2H$	
	$HO(CH_2)_8CH(OH)(CH_2)_8CO_2H$	
	$HO(CH_2)_8CH(OH)CH(OH)(CH_2)_7CO_2H$	
	$HO(CH_2)_8CH=CH(CH_2)_7CO_2H$	
Olea europaea (leaf cutin)	$HO(CH_2)_9CH(OH)(CH_2)_8CO_2H$	50
	$HO(CH_2)_8CH(OH)CH(OH)(CH_2)_7CO_2H$	
Copernicia cerifera (leaf wax)	$HO(CH_2)_nCO_2H$ where n = 17,19,21, ... 29	52, 53
Conifers (leaf wax)	$HO(CH_2)_nCO_2H$ where n = 11,15	54-60
Quercus suber	$HO(CH_2)_{21}CO_2H$	
Betula verrucosa	$HO(CH_2)_8CH(OH)CH(OH)(CH_2)_7CO_2H$	
(cork)	$HO(CH_2)_7CH(OH)CH(OH)(CH_2)_7CO_2H$	38-47
Pseudotsuga taxifolia (bark)	$CH_3CH(OH)(CH_2)_9CO_2H$	48
Abies concolor (bark)	$CH_3CH(OH)(CH_2)_{11}CO_2H$	123
Soybean (seed cerebrins)	$CH_3(CH_2)_{21}CH(OH)CO_2H$	80
Maize (*Zea mays*) (seed cerebrins)	$CH_3(CH_2)_nCH(OH)CO_2H$ where n = 21,23	80

Source	Composition	References
Ricinus communis (seed oil)	$CH_3(CH_2)_5CH(OH)CH_2CH\overset{c}{=}CH(CH_2)_7CO_2H$	61, 64
Dimorphotheca aurantiaca and related spp. (seed oils)	$CH_3(CH_2)_4CH=CHCH=CHCH(OH)(CH_2)_7CO_2H$	77-79
Artemesia absinthium	$CH_3(CH_2)_4CH(OH)CH\overset{t}{=}CHCH\overset{c}{=}CH(CH_2)_7CO_2H$	78
Calliandra eriophylla *Balanites aegyptica* *Cosmos bipinnatus* *Helianthus annuus* (seed oils)	$CH_3(CH_2)_4CH\overset{c}{=}CHCH\overset{t}{=}CHCH(OH)(CH_2)_7CO_2H$	78
Strophanthus spp. (seed oils)	$CH_3(CH_2)_4CH\overset{c}{=}CH(CH_2)_2CH(OH)(CH_2)_7CO_2H$	65, 66
Mallotus philippinensis (seed oil)	$HO(CH_2)_4CH\overset{t}{=}CHCH\overset{t}{=}CHCH\overset{c}{=}CH(CH_2)_7CO_2H$	72-76
Ximenia caffra (seed oil)	$CH_3(CH_2)_3CH\overset{t}{=}CHC\equiv CCH(OH)(CH_2)_6CO_2H$	67, 68
Unguekoa gore (seed oil)	$CH_3(CH_2)_2CH=CHC\equiv CC\equiv CCH_2CH(OH)(CH_2)_6CO_2H$	69-71
Hibiscus abelmoschus (seed oil)	$HO(CH_2)_8CH=CH(CH_2)_5CO_2H$	124
Pharbitis nil (seeds)	$CH_3(CH_2)_2CH(OH)(CH_2)_7CH(OH)CH_2CO_2H$	125
Ipomoea muriatica (seeds)	$CH_3(CH_2)_3CH(OH)(CH_2)_9CH(OH)(CH_2)_2CO_2H$	126
Ipomoea orizabensis (roots)	$CH_3(CH_2)_2CH(OH)(CH_2)_9CO_2H$	127
Ipomoea purga (roots)	$CH_3(CH_2)_4CH(OH)(CH_2)_9CO_2H$	128-130
Desmodium gangeticum (roots)	$CH_3(CH_2)_{13}CH(OH)(CH_2)_2CO_2H$	131
Angelica archangelica (root oil)	$HO(CH_2)_{14}CO_2H$	124
Valeriana officinalis (roots)	$(CH_3)_2CHCH_2CH(OH)CO_2H$	132
Cryptocarya massoia (bark oil)	$CH_3(CH_2)_4CH(OH)CH_2CH=CHCO_2H$	133-135
Lesquerella lasiocarpa (seed oil)	$CH_3(CH_2)_5CH(OH)CH_2CH\overset{c}{=}CH(CH_2)_9CO_2H$	136

Source	Composition	References
MICROORGANISMS		
Pseudomonas pyocyanea	$CH_3(CH_2)_6CH(OH)CH_2CO_2H$ (D-isomer)	89, 90
Pseudomonas viscosa	$CH_3(CH_2)_8CH(OH)CH_2CO_2H$ (D-isomer)	91
Escherichia coli	$CH_3(CH_2)_{10}CH(OH)CH_2CO_2H$ (D-isomer)	92
Lactobacillus casei	$(CH_3)_2CH(CH_2)_nCH(OH)CO_2H$ where $n = 1,2$ (DL-isomers)	93-96
Ustilago zeae	$CH_3(CH_2)_nCH(OH)CH_2CO_2H$ where $n = 2,4$ (L-isomers) $HOCH_2CH(OH)(CH_2)_{13}CO_2H$ (15D-isomer) $HOCH_2CH(OH)(CH_2)_{12}CH(OH)CO_2H$ (2D, 15D-isomer)	102, 103
Yeast (*Saccharomyces cerevisiae*) (cerebrosides)	$CH_3(CH_2)_nCH(OH)CO_2H$ where $n = 21,23$	98, 99
Penicillin mould (unnamed) (cerebrosides)	$CH_3(CH_2)_{21}CH(OH)CO_2H$ $CH_3(CH_2)_{20}CH(OH)CH(OH)CO_2H$	100
Corynebacterium diphtheriae	$CH_3(CH_2)_{12}CH(OH)CH(n\text{—}C_{14}H_{29})CO_2H$ $CH_3(CH_2)_5CH{=}CH(CH_2)_7CH(OH)CH(n\text{—}C_{14}H_{29})CO_2H$	86, 87
Mycobacterium tuberculosis (cord factor)	$(HO)C_{60}H_{120}CH(OH)CH(n\text{—}C_{24}H_{49})CO_2H$	82, 84
Poria tenuis	$CH{\equiv}CC{\equiv}CCH{=}C{=}CHCH(OH)(CH_2)_2CO_2H$	105-108
P. corticola *Basidiomycete* B841	$CH_3C{\equiv}CC{\equiv}CCH{=}C{=}CHCH(OH)(CH_2)_2CO_2H$	

molecular weight acids are also occasionally found, as in the wax of *Juniperus rigida* which contains 24-hydroxytetracosanoic acid[60] and it is probable that other members of the homologous series remain to be discovered in the conifer waxes. Not all conifers appear to contain these acids, at least in appreciable quantities, since Watanabe[57] found their estolides in only half of the 26 species he examined. The corresponding dicarboxylic acids, α, ω-diols, and dihydroxyacids have also been reported in conifer leaf waxes[58, 59].

4.3 *Seeds*

The seed oils of plants usually consist almost entirely of the glycerides of saturated and unsaturated fatty acids. Fatty hydroxyacids are sometimes found which, in view of the positions of the hydroxyl groups along the aliphatic chain, could be intermediates in the biosynthesis of the usual unsaturated acids.

Hydroxyacids are occasionally the principal acid present in the seed oil. As early as 1848 Saalmuller[61] isolated ricinoleic acid from castor oil where it is the major constituent fatty acid combined as triglyceride. Ricinoleic acid has also been found in other seed fats[62, 63]. The determination of the absolute configuration of the hydroxyl group by Serk-Hanssen[64] now allows the assignment of the complete structure of ricinoleic acid as (+)-12D-hydroxyoctadec-*cis*-9-enoic acid.

An analogue of ricinoleic acid, in which the hydroxyl and olefinic groups are transposed, has been isolated from the seed oils of various *Strophanthus* species by Gunstone[65, 66] who suggested that this 9-hydroxyoctadec-12-enoic acid and ricinoleic acid may each be precursors of linoleic acid. However, some of the hydroxyacids which have been isolated from seeds (see Table) do not bear such a close relationship to the common unsaturated acids. Those which have been reported and subsequently shown to be artefacts produced by atmospheric oxidation of unsaturated acids are not included in the table.

Seed oils are found to be sources of some interesting polyunsaturated hydroxyacids. The

oil from *Ximenia caffra,* which contains octadec-*trans*-11-en-9-ynoic acid (ximenynic acid), was found by Ligthelm[67] also to have 3-4% of an analogous hydroxyacid, which Crombie and Griffin[68] identified with synthetic 8-hydroxyximenynic acid. Isanolic acid, an unsaturated hydroxyacid forming 44% of the oil of *Unguekoa gore,* also gives 8-hydroxystearic acid on hydrogenation[69] and from spectroscopic evidence was assumed to be 8-hydroxyoctadec-17-ene-9,11-diynoic acid (8-hydroxyisanic acid)[70]. Seher[71] recently assigned to it the structure 8-hydroxyoctadec-14-ene-10,12-diynoic acid.

The seed fat of the tree *Mallotus philippinensis* is unusual in containing a high proportion of an unsaturated ω-hydroxyacid[72], 18-hydroxyoctadeca-*cis*-9-*trans*-11-*trans*-13-trienoic acid (α-kamlolenic acid)[73–76]. α-Kamololenic acid resembles α-eleostearic acid and isomerizes to the all-*trans* isomer analogous to β-eleostearic acid in ultra-violet light in the presence of iodine.

Certain polyunsaturated hydroxyacids from seed oils are found to be responsible for interference with the hydrogen bromide detection and estimation of naturally-occurring epoxy acids[77, 78]. The principal one of these, 9-hydroxyoctadeca-*trans*-11-*trans*-13-dienoic acid (dimorphecolic acid), is found in *Dimorphotheca aurantiaca*[79] and related species[77, 78]. Epoxyacids and such hydroxydienoic acids can now be separately estimated by hydrogen bromide titration before and after lithium aluminium hydride reduction which reduces the epoxy-compounds and leaves the hydroxyacids unchanged[77].

In addition to the oils, plant seeds contain lipids not unlike the cerebrosides of animal tissue, derived not from sphingosine but from phytosphingosine (3).

$$CH_3(CH_2)_{13}CH(OH)CH(OH)CH(NH_2)CH_2OH \qquad (3)$$

The phytosphingosine-containing lipid from soybean contains α-hydroxytetracosanoic acid, while that from maize yields a mixture of the C_{24} and C_{26} α-hydroxyacids[80].

5 HYDROXYACIDS FROM MICRO-ORGANISMS

Some of the lipids produced by microorganisms exhibit biological activity with the result that they have been thoroughly investigated. Hydroxyacids are commonly found as constituents of these lipids.

5.1 *Bacteria*

The most intensively-studied bacterial lipid is that from the tuberculosis bacillus, *Mycobacterium tuberculosis*. The organism contains up to 40% dry weight of wax which is responsible for its resistance to drying and to chemical attack and also for its virulence and characteristic tendency to aggregate in cord-like colonies. The fraction of the wax which causes the organism to form cords (the cord factor) contains mycolic acid, a dihydroxyacid of high molecular weight first isolated by Anderson and co-workers[81] in 1938. It has been shown by Asselineau and Lederer[82] to be a β-hydroxyacid with a long side-chain in the α-position (4). The second hydroxyl group has yet to be located.

The cord factor itself has been shown to be trehalose-6,6′-dimycolate[83]. Several mycolic acid analogues have been isolated from different strains of the bacterium[84].

β-Hydroxyacids with long side-chains have also been isolated from the diphtheria bacillus, *Corynebacterium diphtheriae*[85]. Corynomycolic acid was shown by Lederer and co-workers to be (+)-2-tetradecyl-3-hydroxyoctadecanoic acid[86] and a Δ^{11}-unsaturated analogue was also identified[87]. In studies of the biosynthesis of these compounds it was shown[88] that diphtheria bacilli incubated with 1-^{14}C-palmitic acid produced corynomycolic acid labelled only at carbon atoms 1 and 3, establishing its synthesis from two molecules of palmitic acid (Figure 4). A similar scheme was postulated for the biogenesis of the higher mycolic acids

$$(HO)C_{60}H_{120}CH(OH)CH(n\text{-}C_{24}H_{49})CO_2H \qquad (4)$$

from stearic acid and hexacosanoic acid, both of which are found in tubercle wax.

$$C_{15}H_{31}CO_2H + (C_{14}H_{29})CH_2CO_2H \rightarrow C_{15}H_{31}COCH(C_{14}H_{29})CO_2H$$
$$\rightarrow C_{15}H_{31}CH(OH)CH(C_{14}H_{29})CO_2H$$

Figure 4

Bergstrom, Theorell, and Davide[89] have isolated (—)-3-hydroxydecanoic acid from the mixture of acids produced on hydrolysis of pyolipic acid, a complex lipid produced by *Pseudomonas pyocyanea* which is bacteriostatic towards *Mycobacterium tuberculosis* and toxic to mice. When the organism is grown on media containing glycerol the antibiotic is obtained as a crystalline compound of the hydroxyacid with L-rhamnose[90]. *P. viscosa* produces an antibiotic, viscosin, which is an acidic polypeptide hydrolysable to a mixture of amino-acids and 16 to 18% of oily fatty acids consisting of dec-2-enoic acid and (—)-3-hydroxydecanoic acid[91]. The unsaturated acid is believed to be produced from the hydroxyacid by dehydration during hydrolysis.

The bacillus *Escherichia coli* produces an agent capable of causing regression in an experimental mouse tumour which appears to be a polysaccharide containing peptide and phospholipid components. The phospholipid moiety contains (—)-3-hydroxytetradecanoic acid in addition to normal saturated fatty acids[92].

Camien and Dunn[93–95] have shown that αD-hydroxyacids are essential for the growth of *Lactobacillus casei* and are normally produced by the organism by racemization of αL-hydroxyacids. DL-lactic acid, together with smaller amounts of αDL-hydroxyisopentanoic and αDL-hydroxyisohexanoic acids were found among the metabolic products of *L. Casei*[96]. A mutant incapable of effecting this racemization provided a bioassay for the growth-promoting effects of hydroxyacids. The activity of αD-hydroxyacids was found to increase with chain length, suggesting that the ultimate need was for a long-chain acid similar to those which occur in cerebrosides. Since αL-hydroxyacids were not growth-promoting, the mutant strain of *L. casei* served as a means of confirming that the α-hydroxyacids of cerebrosides and wool wax have the D-configuration. There was some indication that the hydroxypalmitic acid reported to be present in butterfat[97] may also be a αD-hydroxyacid.

5.2 *Fungi*

The common yeast, *Saccharomyces cerevisiae,* produces a lipid material which contains a fraction analogous to the cerebrosides of animal tissue. Reindel[98] hydrolysed yeast cerebrin and identified 2-hydroxyhexacosanoic acid and phytosphingosine in the products. Chibnall, Piper, and Williams[99] claim that 2-hydroxytetracosanoic acid is also present.

Two cerebrins isolated from a penicillin-producing mould (identity not stated) have been hydrolysed to give 2-hydroxytetracosanoic acid and 2,3-dihydroxytetracosanoic acid respectively, and phytosphingosine[100]. The yeast *Torulopsis magnoliae,* when incubated in culture media, produces large amounts of an oil heavier than water which settles out of the medium. The oil is a mixture of compounds of sophorose with hydroxyacids which have been identified as 17-hydroxystearic and 17-hydroxyoleic acids[101].

The corn smut organism *Ustilago zeae* produces an antibiotic metabolite, ustilagic acid, which is hydrolysed to a remarkable mixture of compounds including several hydroxyacids. Alkaline hydrolysis of ustilagic acid, which proved to be a mixture, afforded acetic acid, (+)-3-hydroxyhexanoic acid, (+)-3-hydroxyoctanoic acid, a small amount of hexanoic acid, and glucoustilic acid[102]. Acid hydrolysis of the last gave two acids, identified as 15D,16-dihydroxyhexadecanoic acid and 2D,15D,16-trihydroxyhexadecanoic acid[103], after their separation from one another by virtue of the formation of the neutral diisopropylidene derivative of the trihydroxyacid[104] (Figure 5).

Two unsaturated hydroxyacids and their lactones isolated from cultures of *Poria tenuis, P. corticola,* and an unnamed Basidiomycete are reported to have antibiotic activity[105, 106]. They have been identified as 4-hydroxyundeca-5,6-diene-8,10-diynoic acid, 4-hydroxydodeca-5,6-diene-8,10-diynoic acid, and the corresponding lactones[107, 108].

6 SOME BIOCHEMICAL OBSERVATIONS

The α-hydroxyacids, in combination with sphingosine in animal tissues or with phytosphingosine in plants and microorganisms, are apparently constituents of all living organisms. The functions they perform and the methods by which they are synthesized are almost entirely unknown.

$HOCH_2CH(OH)(CH_2)_{13}CO_2H$
Ustilic acid A

$HOCH_2CH(OH)(CH_2)_{12}CH(OH)CO_2H$
Ustilic acid B

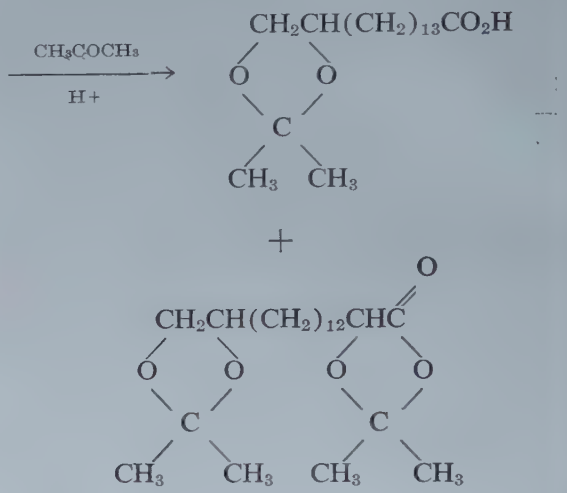


Figure 5

In brain and nerves the cerebrosides are associated with cholesterol and phospholipids forming the myelin sheath. The myelin lipids, unlike other lipids in the body, are not subject to rapid metabolic turnover. Having once been laid down they seem to be resistant to change. Thus brain homogenates from 15-day-old rats incorporated 1-^{14}C-acetate and 1-^{14}C-palmitate, while those from 24-day- and 42-day-old in which myelination is known to be complete did not[109]. Where the labelled material was incorporated into phrenosin it was found entirely in the fatty acid moiety. Intravenous injection of labelled cerebrosides into mature monkeys gave rise to radioactivity in various organs but not in the brain[110]. This immobility of the myelin lipids is claimed to be a reflection of their structural role[111]. However, where the cerebrosides occur in other organs, in plants and in micro-organisms their purpose may not be structural, but that they are important is suggested by the apparent requirement for the constituent D-α-hydroxyacids by a *Lactobacillus casei* mutant which lacked the ability to synthesize its own[93–95].

The occurrence of α-hydroxyacids in wool wax is also of interest in biogenesis, particularly in view of the presence of a large proportion of branched-chain members. Shorland[112] has suggested that branched-chain fatty acids produced by ruminant animals might arise by acetate addition to low molecular weight branched acids resulting from deamination of amino-acids in the rumen. Camien, Fowler, and Dunn[96] have shown that branched-chain α-hydroxyacids are metabolic products of *Lactobacillus casei,* and since the alimentary tract of animals, and particularly that of ruminants, is richly populated with micro-organisms, these are a possible alternative source of the branched-chain acids which are found in animal fats, and more particularly, the branched-chain α-hydroxyacids present in wool wax.

The biological sources from which α-hydroxyacids were isolated are those which are generally noted for their relatively high content of sterols. One might therefore speculate whether some metabolic connection exists between these two classes of lipid. It has for instance been demonstrated that the feeding of cerebrosides or cerebroside fatty acids to animals causes increased metabolic turnover of cholesterol and its increased excretion in the faeces[113–115]. Other hydroxyacids also show activity in the animal gut. The purgative action of castor oil is attributed to liberated ricinoleic acid and the diarrhea often associated with steatorrhea is paralleled by increased excretion of the 10-hydroxystearic acid produced by intestinal microflora[37].

A high proportion of the naturally occurring hydroxyacids have been discovered through

investigations of biologically active material. The data accumulated in this way is scattered but there seems to be sufficient evidence of the biological importance of fatty hydroxyacids to warrant further study.

7 METHODS OF INVESTIGATION

7.1 *Detection*

The presence of hydroxyacids in mixtures of fatty acids can be detected readily by determination of the saponification equivalent of the acids or their esters before and after acetylation. In spite of this simple and accurate procedure the occurrence of hydroxyacids in common lipids has often been overlooked. Examination of the region near 3300-3400 cm^{-1} in the infra-red spectrum of the esters of fatty acid mixtures affords a more rapid and convenient method of detection which is well suited as a screening procedure and as a means of following the separation of hydroxylated and unhydroxylated esters by adsorption chromatography.

7.2 *Isolation*

The initial step is usually the saponification of naturally occurring esters or the acid hydrolysis of amide or glycosidic linkages, and this can normally be accomplished by any of the well-known procedures. Some materials such as wool wax have proved more intractable but Barnes, Curtis, and Hatt[116] have defined conditions under which such materials can be saponified rapidly with ethanolic alkali by proper choice of concentrations. This method also facilitates the subsequent extraction of the unsaponifiable material. The usual procedure, following saponification and removal of the unsaponifiable material, was to liberate the acids by acidification of the solution of their alkali metal salts. However, Barnes[117] has shown that in the presence of ethanol this can lead to appreciable esterification of the liberated acids. When hydroxyacids are present, inter- and intra-esterification of these must also occur. This has been avoided in studies of the acids of a number of lipids by precipitation of the calcium salts of the acids by addition of calcium chloride to their alkaline solutions, followed by refluxing the calcium salts with an appropriate alcohol and excess mineral acid to form the esters of the fatty acids[17, 26, 53].

Methods involved in the subsequent examination of the acids include crystallization, distillation, adsorption and partition chromatography, counter-current distribution and gas chromatography. The use of these methods for the separation and analysis of fatty acids has recently been reviewed[118].

In earlier work, crystallization was virtually the only method available for the isolation of the hydroxyacids. Usually only the most insoluble or the most abundant compound present was obtained and then often in an impure condition. Due to the development of better methods, particularly low-pressure distillation and gas chromatography, it is now recognized that, in common with most natural aliphatic compounds, the hydroxyacids tend to occur in homologous series, so that crystallization can no longer be regarded as a useful isolation procedure.

In recent work the initial separation of the hydroxyacids from unhydroxylated acids was usually accomplished by adsorption chromatography of their methyl esters, as in the investigations of carnauba wax[53], wool wax[26] and beeswax[30], or by counter-current distribution, as in the study of wool wax α-hydroxyacids by Horn and co-workers[25], of cutin hydroxyacids by Matic[49] and of the acetylenic hydroxyacids of fungi by Bu'Lock, Jones and Leeming[107, 108]. In order to avoid the hydrolysis which can occur during the adsorption chromatography of esters, Downing, Kranz, and Murray reduced the esters of wool wax[26] and carnauba wax acids[53] and chromatographed the resulting mixtures of mono- and di-hydric alcohols. The α, β-diols arising from the α-hydroxyacids of wool wax were made more readily separable from the α, ω-diols derived from the ω-hydroxyacids by conversion to their isopropylidene derivatives. A similar procedure should be suitable for the separation of the reduction products of β-hydroxyacids.

Subsequent investigation of the individual members of mixtures of hydroxyacids is now usually achieved by low-pressure distillation or gas chromatography of their esters or other derivatives. Murray and Schoenfeld[52] found it possible to distil the acetylated methyl esters of carnauba wax ω-hydroxyacids up to chain lengths of 30 carbon atoms, while Horn and Pretorius[27] were able to distil the C_{30} and C_{32}

ω-hydroxyacids of wool wax in the form of their cyclic lactones. The acetylated methyl esters of the wool wax α-hydroxyacids were much more thermolabile and decomposition became serious at the distillation temperature of the C_{18} acid derivatives[25]. These were investigated by distillation of the derived diol acetates. Fatty acids having secondary hydroxyl groups isolated from the carboxyl group could be expected to be somewhat more stable than the α- and β-hydroxyacids. However, the inaccessibility of the higher molecular weight compounds is a serious limitation of the method which is tedious, requires relatively large quantities of material and is unsuited to the isolation of the minor constituents of aliphatic mixtures.

For analysis by gas chromatography, hydroxyacids must be converted to some reasonably stable derivative. Kishimoto and Radin[16] were able to examine the α-hydroxyacids of rat-brain cerebrosides up to a chain-length of 26 carbon atoms in the form of the methyl ester methyl ethers. In the investigations of the hydroxyacids from wool wax[26], beeswax[30], carnauba wax[53] and sheep brain[17] it was found convenient to convert the acids to the corresponding hydrocarbons. This allowed the investigation of compounds having up to 36 carbon atoms and greatly reduced the number of reference compounds required.

7.3 *Structure determination*

The chemical investigation of the structure of hydroxylated fatty acids is usually relatively simple provided the compound under study is a pure material. The position of a hydroxyl group or a carbon-carbon multiple bond is fairly readily determined by oxidative fission of the molecule and identification of the fragments. When the hydroxyl group is situated in the α-, β-, or ω-positions the fact is readily established by the simple reactions of the compound, which may also be applied to mixtures of similarly-substituted acids. The greatest confusion has always occurred in the study of compounds which were believed pure but which actually were mixtures. This situation has occurred with the α-hydroxyacids from cerebrosides. The position of the hydroxyl group was established with little difficulty but rival claims regarding the presence of a single C_{24} α-hydroxyacid or, alternatively, of a mixture of the C_{22}, C_{24}, and C_{26} compounds, extended into the present decade[14], whereas at least thirteen such saturated acids are now known to occur in the material[17]. Uncertainty regarding the constitution of the hydroxyacids of cork was rather more serious and phellonic acid was successively regarded as 2-hydroxydocosanoic acid[39], 22-hydroxytetracosanoic acid[40] and 22-hydroxydocosanoic acid[41].

It is in the chain-length analysis of homologous series that gas chromatography has been of most service in the study of hydroxyacids but with the development of new methods it has begun to be of greater value in structure determinations. Consideration of relative retention times has provided evidence for the presence and position of methyl side-chains in the hydroxyacids of wool wax. The use of a molecular sieve in gas chromatography is found to give conclusive evidence regarding the presence of chain branching in the hydrocarbons derived from hydroxyacids[26] and determination of the position of chain branching can now be made by gas chromatographic examination of the products of oxidative degradation[119]. Hydroxyacids are also amenable to structure determination by mass spectrometry and the use of this tool in lipid research has been reviewed recently[120].

8 INDUSTRIAL APPLICATIONS

The only naturally occurring hydroxyacid which appears to have attained significant industrial utilization is ricinoleic acid, due largely to the fact that castor oil can be produced cheaply and contains 80% of the hydroxyacid which for many purposes need not be separated from the remainder of the fatty acids. Dienoic acids produced by dehydration of ricinoleic acid are used in the manufacture of paints; 12-hydroxystearic acid prepared by hydrogenation of the unsaturated acid is used in lubricants in the form of metallic salts; dibasic acids produced by oxidative fission of ricinoleic acid are used in the manufacture of a form of nylon and of plasticizers for polyvinyl chloride resins.

There seems little doubt that if other hydroxyacids could be produced economically they also would have industrial uses, either by virtue of their physical properties or in specific

applications depending on the position of the hydroxyl group. There has been some interest in the ω-hydroxyacids in view of the musk-like odour of the cyclic lactones having 14 to 18 carbon atoms and the valuable perfume bases dihydrocivetone and exaltone have been synthesized from kamlolenic acid[121].

Probably the largest untapped source of hydroxyacids is in wool wax, the acid fraction of which contains 30% of α-hydroxyacids and 5% of ω-hydroxyacids. It has been found[122] that certain wool wax α-hydroxyacid esters are useful secondary plasticizers for polyvinyl chloride resins and that the ω-hydroxyacids could be utilized in the production of hard waxes. Economic exploitation of these acids, however, depends largely on the advantageous disposal of the remainder of the wax.

9 CONCLUSION

While methods have now become available for the fuller investigation of hydroxyacids from natural sources, further refinements in technique are necessary, particularly for the isolation of individual components from homologous series in sufficient quantity and purity for structure determinations. The widespread occurrence of hydroxyacids, their structural role in tissues and their increasingly frequent isolation from biologically active material will undoubtedly provide the incentive for further study.

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Hydrogen Bonding in Ortho Substituted Primary Aromatic Amines

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1 INTRODUCTION

Many chemists have assumed that there will be intramolecular hydrogen bonding in some *ortho*-substituted anilines similar to that which has been demonstrated to exist in the corresponding phenols. Rodebush[1], who was one of the two proposers of the term 'hydrogen bond'[2], stated in 1941 that in this connection 'amines have proved a very disappointing material for infra-red study'. When species such as *o*-nitroaniline had failed to show effects due to intramolecular hydrogen bonding of the magnitude expected, it was suggested[3,4] that such bonds were usually weak and in some cases, such as that of *o*-nitroaniline, they were not significant. This has resulted in a number of investigations which have sought to prove that such bonds exist and to obtain some measure of their strength. The time is opportune for a critical review of the present status of the problem.

2 GENERAL ASPECTS OF HYDROGEN BONDING

2.1 *Defining the hydrogen bond*

It is often considered to be essential that any discussion should commence by definition of the terms to be used. Pimentel and McClellan in their recent book[5] state 'a hydrogen bond exists between the functional group A–H and an atom, or group of atoms, B in the same, or a different, molecule when

(a) there is evidence of bond formation (association or chelation)

(b) there is evidence that this new bond linking A–H and B specifically involves the hydrogen atom already bonded to A.'

This definition merely shifts the decision to the question of what is to be considered as sufficient evidence of association or chelation. Like other definitions in science it suffices to distinguish cases that are well removed from the boundary that the definition seeks to set up. It becomes a matter of convention, or arbitrary decision, as to which side of the boundary we choose to place many of the intermediate cases. We speak of van der Waals bonds holding the units of a molecular crystal in their appropriate positions in the lattice. The lattice is stable and the chemist who, since the invention of the term valence, has a predisposition to invent 'bonds' as the images of the restraints which maintain ordered structures, can allocate specific energy contributions to the 'bonds' holding a unit to its nearest neighbours. Chemists have not been disposed to speak of a bond between two such molecules when they collide in the gaseous state at higher temperatures. Under these conditions the van der Waals attraction is much less than the mean energy of collision *(kT)* and the colliding pair cannot retain their relationship for one vibration period, a time of the order of 10^{-13} sec. In this brief encounter the molecules will have passed through a condition of attraction as strong as that which existed in the crystal. If such collisions are frequent there will be an appreciable distortion of the atomic or molecular spectrum and it will be a matter of choice whether we regard the pair as a van der Waals dimer, or as separate molecules in collision. A similar situation can arise in our decision under section (a) of the above definition. Pure aniline has two broad bands in the infra-red absorption spectrum at 3355 and 3429 cm^{-1} corresponding respectively to the in-phase (symmetric) and out-of-phase (antisymmetric) vibrations (1) and (2).

As the amine is diluted with a non-polar solvent, such as carbon tetrachloride, these bands become sharper and move to higher frequencies[3,6], until, in solutions more dilute than 0.1*M*, they remain constant at 3395 and 3482 cm^{-1} (Figure 1). At no concentration is there a doubling of the frequencies similar to that which occurs in phenols, where it indicates an equilibrium between a monomer and a hydrogen bonded dimer. Under these conditions it is not possible to decide how much of the lowering of frequency and broadening of the amine absorption bands is due to transient hydrogen bonding or to other polar interactions. The latter are expected to be stronger between the polar, and polarizable, NH_2 groups than for the rest of the bonds in the molecule. Some of the differences of interpretation of experimental results in connection with *ortho* substituted amines have depended on making differentiations of this sort. Dyall and Hambly[3] chose to regard the variation in frequency of the NH vibrations of aniline as due to general polar interaction. They only considered as significant evidence of intramolecular hydrogen bonding, changes in amine frequency produced by adjacent substituents which were greater than could be produced by molecular interaction without the formation of specific complexes.

Others[7,9] have treated the departure of the NH frequencies in *ortho* compounds from a relationship found to apply to *meta* and *para* substituted amines[10] as evidence for the formation of a hydrogen bond. Any direct polar interaction under these conditions would not be symmetrical and could be defined as a hydrogen bond. Bellamy and Williams[10] used the conformity of pure aniline to the general relationship for dilute, non-polar solutions of *meta* and *para* substituted anilines as evidence against the existence of intermolecular hydrogen bonding.

Normal chemical conventions are satisfied if a determination of crystal structure, or other diffraction study, demonstrates a special relationship between the atoms A and B which are held by a hydrogen bond. Equally acceptable evidence is provided by spectra which demonstrate an equilibrium between two structures which have the characteristics expected for the bonded and non-bonded forms. The *m*- and *p*-halogen substituted phenols show only a single OH absorption band in

very dilute carbon tetrachloride solutions, but the corresponding *ortho* compounds show doubled absorptions both in the fundamental and overtone regions of the infra-red spectrum.

2.2 *The electronegativity criterion for hydrogen bonding*

The dictum of Pauling[13] that 'only the most electronegative atoms should form hydrogen

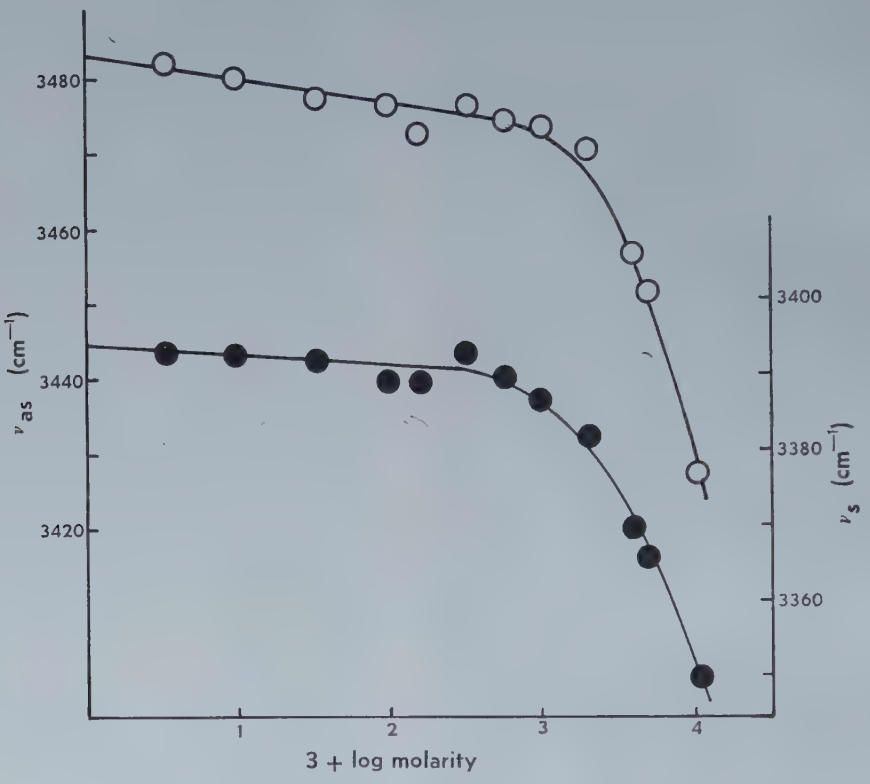


Figure 1 *Variation of the N–H stretching frequencies of aniline during dilution with carbon tetrachloride. Open circles, ν_{as}; filled circles ν_s*

The relative intensities of the members of a double band do not change on dilution so they cannot arise from intermolecular bonding, but they do change with temperature as required for an equilibrium between isomers. The absorptions have been attributed[11] to a *cis* chelated structure and a *trans* structure in which there is no intramolecular hydrogen bond. The absorption band is single when similar halogen atoms are present in the 2 and 6 positions to hydroxyl, but is again double[12] when different halogen atoms are present at positions 2 and 6. Other types of evidence may be indicative of hydrogen bonding but are not so satisfactory.

bonds and the strength of the bond should increase with increase in the electronegativity of the two bonded atoms' is often quoted, but it unduly simplifies the relationship between hydrogen bonding and the electronegativity of the bonded atoms. Positively charged ions are more electronegative than uncharged atoms, but we do not find hydrogen bonds between two such units, though ammonium or hydronium ions may act as the hydrogen donor in bond formation with a less electronegative unit. The hydrogen bond in the HF_2^- ion, where the electronegatively of fluorine has been reduced by the extra electron, is estimated to be some ten times stronger than the hydrogen bond

between normal fluorine atoms in hydrogen fluoride polymers. The roles of hydrogen donor and hydrogen acceptor may be played by the same atomic species, but, except in the rare cases in which a symmetrical bond is formed, two different types of electron interaction are concerned. The electronegativity of the hydrogen donor atom, A, must be sufficiently high so that its bond to hydrogen becomes polarized and the exposed proton can attract strongly the electrons in a 'lone pair' orbital of the acceptor atom, B. If the electronegativity of the acceptor atom is too high it will prevent the polarization of the lone pair orbital and so inhibit strong bonding to the proton. On the other hand if the electronegativity of the donor atom is too high and the polarizability of the lone pair orbital on the acceptor is also high, there will be an acid-base reaction and the proton will transfer from A to B. Because, in this case, hydrogen bonding passes over into salt formation, Pimentel and McClellan[5] speak of the donor as the acidic group and the acceptor as the basic group. A narrow range of electronegativities is therefore required for the formation of strong hydrogen bonds. There are examples among the *ortho* substituted anilines where a reduction of the electronegativity of the acceptor atom produces stronger hydrogen bonds. (See section 4.3.) In so far as the hydrogen bond is regarded as electrovalent in nature, and due to a proton located between two negatively charged atoms, the bonding is similar to that of triple ions such as ($Cl^-Na^+Cl^-$) which are believed to be present in concentrated electrolyte solutions. It also has a covalent nature involving a molecular orbital built from the 1*s* atomic orbital of hydrogen and appropriate orbitals of the donor and acceptor atoms. In this it resembles the three centre orbitals of the bridge atoms in boron hydrides.

3 DETECTION OF INTRAMOLECULAR HYDROGEN BONDS

3.1 *Effects on simple physical properties*

Badger[14] has given a general review of the methods for detection of intramolecular hydrogen bonds; attention in the present review will be confined to their application to primary amines and to closely related species from which inferences can be made. He has emphasized that hydrogen bonds, which can only be detected by small changes in the infra-red spectrum and do not change physical and chemical properties appreciably, are of little significance.

When suitable donor and acceptor groups are present as substituents in a benzene derivative, intermolecular hydrogen bonding may occur between molecules of the *meta* or *para* isomers while intramolecular bonding can occur, if there is a suitable geometric arrangement, in the *ortho* derivative. When the donor group is the primary amine, it is necessary to consider the possibility that one of the NH groups could participate in intramolecular bonding to an *ortho* substituent and the other to the acceptor group in an adjacent similar molecule or to a suitable solvent molecule. The conditions are therefore more complex than those experienced with phenols, secondary amines and anilides, and the interpretation of observations is correspondingly more difficult.

Intermolecular hydrogen bonding will increase the lattice energy in the solid state and will control the order in the lattice structure, so causing the energy and entropy of sublimation for *meta* and *para* derivatives to exceed that of the *ortho* isomer with its intramolecular hydrogen bond. Some of the intermolecular hydrogen bonding may be maintained in the liquid phase, and only lost on evaporation, so that there will be differences in the latent heats and entropies of evaporation. The intermolecular bonds frequently, but not always, raise the density, the melting point and the boiling point. The increase in molecular size and intermolecular attractions raise the viscosity above that of the chelated isomer. The data of Table 1 provide little confirmation for the claim of Pimentel and McClellan (reference 5, p. 189) that intramolecular hydrogen bonding occurs in *o*-chloroaniline. The entropies of evaporation at the normal boiling point, for all the compounds listed, are higher than the normal value predicted by Trouton's rule and suggest that unusually strong intermolecular attraction (hydrogen bonding?) occurs in all cases.

When hydrogen bonding is strong we expect to find large differences between the solubility of *ortho* isomers on the one hand and

the *meta* and *para* isomers on the other, in solvents which cannot form hydrogen bonds. When the hydrogen bonding power of the solvent is sufficiently powerful, either in donation or acceptance, to overcome the intramolecular bonding of the *ortho* compound there will be similarity in solubility. Albert and Goldacre[16] inferred the presence of an intramolecular hydrogen bond in 1-aminoacridine from the difference between its behaviour and that of its isomers towards solvents. The wet melting point method of Baker[17] depends on the lower solubility of the *ortho* isomer in water, when the chelate bond is sufficiently strong to resist the alternative hydrogen bonding to water. The melting point of the intramolecularly bonded compound is only slightly depressed when the sample is thoroughly wetted, but the melting points of the *meta* and *para* isomers are strongly depressed. Chaplin and Hunter[18] found for *o*-nitroacetanilide a depression of 10°C in the melting point of the wet sample, whereas the depression was 51°C for the *para* isomer. The differences between the wet and dry melting points for *ortho* and *para* nitroaniline, 9°C and 24°C respectively[6], are less pronounced and the result of this rather crude test must be interpreted with caution.

Table 1 Effects of possible hydrogen bonding on physical properties

Compound	M.P. (°C)	B.P. (°C)	ΔH_{evap} kcal mole^{-1}	$\Delta S°_{evap}$ cal deg^{-1}	Viscosity (mP)	Density (g cm^{-3} at20°C)
2-nitrophenol	45	210	12·61	26·1	6·23 (150°C)	1·447
3-nitrophenol	97	194 (70 mm)	—	—	—	1·485
4-nitrophenol	114	279d.	—	—	20·80 (150°C)	1·468
2-nitroaniline	72	284·1	15·27*	27·4	9·2 (172°C)	1·442
3-nitroaniline	114	306·4	15·75	27·2	9·4 (172°C)	1·430
4-nitroaniline	147	331·7	18·49	30·6	14·4 (172°C)	1·424
2-chloroaniline	0	210·5	11·12	23·1	3·40 (210°C)	1·213
3-chloroaniline	−10	229·8	12·02	23·9	—	1·216
4-chloroaniline	71	231·0	12·90	25·6	3·91 (210°C)	—

* Hoyer and Peperle[15] give values for the internal heats of sublimation of *o*, *m* and *p*-nitroanilines as 20·9, 22·7 and 28·5 kcal mole^{-1} respectively.

3.2 *Molecular weight in solution*

One of the more direct methods for indicating chelate hydrogen bonding is to compare the molecular weights of *ortho*, *meta* and *para* isomers as determined by depression of freezing point of a solvent. The solvent, usually benzene or naphthalene, is only a very weak acceptor of hydrogen bonds. Hunter[18,19], who has made extensive investigations by this method, has shown that the apparent molecular weight of the solute increases rapidly with concentration when the substituent groups form intermolecular bonds, while the apparent molecular weight of the chelated *ortho* compound shows little variation with concentration. Although he did not study simple nitroanilines he made a full investigation of their acetyl and benzoyl derivatives. His results for the nitroacetanilides agreed with the prediction from the wet melting point determination, that there was chelation in the *ortho* compound. When a methyl group was present in the 6 position of a 2-nitroacetanilide the acetylamino group was forced out of the plane of the ring and intermolecular, rather than intramolecular, hydrogen bonds were indicated by the molecular weight determination.

3.3 *Polarographic half-wave potentials*

Astle and co-workers[20–22] introduced the study of polarographic reduction potentials for demonstrating the effects of hydrogen bonding to a nitro group. They found that the separation into two stages of the reduction process for nitrophenols could be interpreted satisfactorily in terms of hydrogen bonding both in the initial nitro compound and in the *ortho*-hydroxyphenyl hydroxylamine which is the first reduction product. Runner and Wagner[23]

found that in media more acid than pH 12 *ortho*-nitroacetanilide is reduced more readily than its *meta* or *para* isomers and more readily than nitrobenzene. They attributed this to the joint influence of the acetyl group in reducing the electron density at the nitro group and to the electron attraction produced by the internal hydrogen bond. They did not regard the method as diagnostic for such a bond, but thought it necessary to obtain other evidence as partial confirmation. They reported that the relative rates of sublimation at 228°C of the *o*-, *m*-, and *p*-nitroacetanilides were 32:3:1,

Holleck and co-workers[24,25] claim to detect chelate bonding to nitro and aldehyde groups by departure of the half wave reduction potential from a relationship that is found to hold for other *ortho* substituted nitrobenzenes and benzaldehydes. They present their results by setting out the half wave reduction potentials, extrapolated to pH=0, for the *para* derivatives along a line of arbitrary slope (Figures 2, 3). The position of the substituent on this line gives the ordinate for plotting the corresponding reduction potentials of the *meta* and *ortho* derivatives. They find constant values of $\epsilon_{\frac{1}{2}}$ for all *meta* derivatives, and a linear relationship for all *ortho* derivatives where no hydrogen bond is expected before reduction. The deviations found, in those cases where hydrogen bonding was predicted, are in opposite directions in the two series. This could be attri-

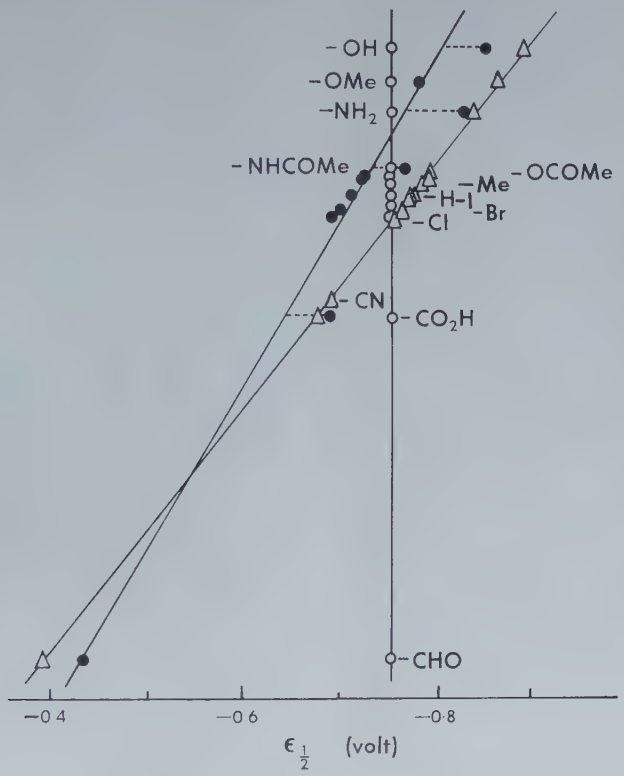


Figure 2 *Half wave potentials, $\epsilon_{\frac{1}{2}}$, for the polarographic reduction of substituted benzaldehydes*† (*reference* 25). ● Ortho; ○ meta; △ para

† The points are plotted in the figure according to reference 25. These do not correspond exactly to the values tabulated in reference 24. If the latter are replotted on the same potential scale as in Figure 3, but with exclusion of the data for the dialdehydes, the values for *ortho* substituents do not follow a linear relationship. The legends for the figures of reference 25 which correspond to figures 2 and 3 have been interchanged.

buted to a reduction in the bond order of C=O as a result of chelation, while if chelation affects one of the N—O linkages of the

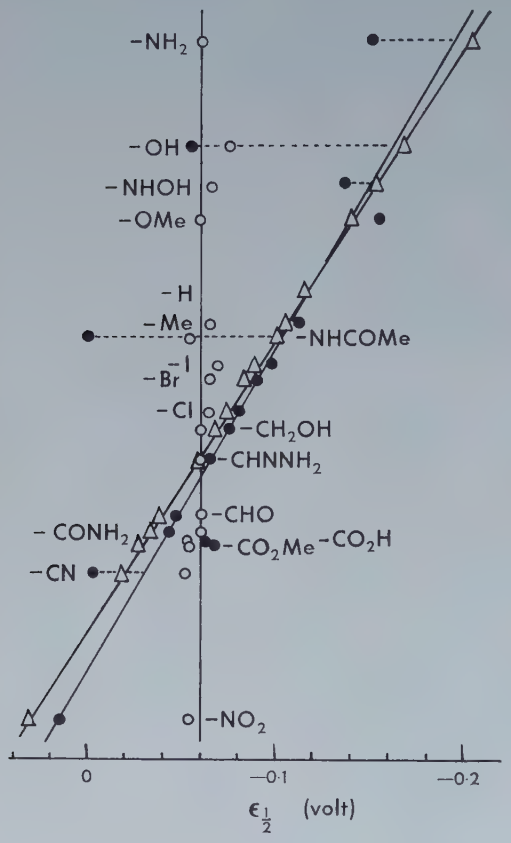


Figure 3 *Half wave potentials, $\epsilon_{\frac{1}{2}}$, for the polarographic reduction of submitted nitrobenzenes (reference 25).* ● Ortho; ○ meta; △ para.

nitro group the bond order of the other would be increased. However, the results are open to question. The three lines for *ortho, meta* and *para* derivatives should intersect at the point characteristic of the unsubstituted benzaldehyde or nitrobenzene; the deviation from this requirement gives a measure of the uncertainty of the linear relationships. The influence of substituents follows a different order in the two series, and there seems to be a statistical factor which makes the dialdehyde and dinitro compounds take extreme values of $\epsilon_{\frac{1}{2}}$. Particularly in the series of aldehydes, the slope of the line for *ortho* substituents is dominated by this point. Where similar compounds have been studied by other workers[20] the corresponding values of $\epsilon_{\frac{1}{2}}$ differ by greater amounts than the deviations attributed to hydrogen bonding. If the results of Holleck

Table 2 *pK_a values for nitronaphthylamines in water at 25°C*

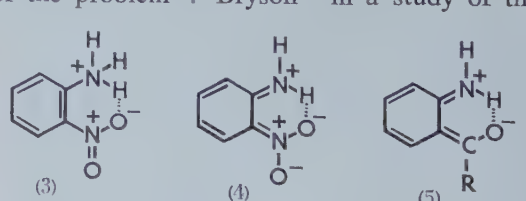
Substituents	pK_a	Substituents	pK_a
1-amino-3-nitro	2·07	2-amino-4-nitro	2·43
1-amino-4-nitro	0·54	2-amino-5-nitro	3·01
1-amino-5-nitro	2·73	2-amino-6-nitro	2·62
1-amino-6-nitro	2·89	2-amino-7-nitro	3·10
1-amino-7-nitro	2·55	2-amino-8-nitro	2·73
1-amino-2-nitro	—1·74*	2-amino-1-nitro	—0·85*
1-amino-8-nitro	+2·79	2-amino-3-nitro	+1·48

*The two negative values are derived from measurements in 30·3% sulphuric acid solutions with allowance for the acidity function H_0 of this solution.

et al. can be confirmed, the persistence of chelation for 2-aminobenzaldehyde and 2-nitroaniline in acidic aqueous solution would indicate that the intramolecular bonds of their conjugate acids are much stronger than those of the original bases.

3.4 *Dissociation constants*

The effects of intramolecular hydrogen bonding in an undissociated carboxylic acid and its anion, on the value of its dissociation constant are usually easy to recognize and interpret (reference 13, p. 479, and reference 26). The effects of *ortho* substituents on the pK_a values of anilinium ions cannot be as readily assessed. While the $-NH_3^+$ group of (3) is a good donor for hydrogen bond formation, the effect of a positive pole at the *ortho* position will greatly reduce the capacity of the nitro group to accept such a bond. It will depend on the relative values of these two opposing factors in the free base (4) and its conjugate acid (3) whether the acid will be strengthened or weakened by chelation. *Ortho* effects other than hydrogen bonding contribute to the complexity of the problem[27]. Bryson[28] in a study of the



(3) (4) (5)

dissociation constants of naphthylamines in which he believes, on other grounds, that there is chelation between nitro and amino groups,

found that three show very low base strength but the fourth has a normal value for a nitroamine (Table 2). At the present stage it appears to be more appropriate to discuss the contribution of chelation to dissociation constants of amines after the existence of such a bond has been demonstrated by other methods.

3.5 *Chromatographic separation*

Hoyer[29] in an extensive study of the chromatographic behaviour of hydroxyanthraquinones was able to show that those which only contained hydroxyl groups in positions where they could form a six-membered chelate ring to carbonyl, passed rapidly through columns packed with silicic acid or alumina when in benzene solution. The hydroxyanthraquinones in which chelation of some of the hydroxyl groups was sterically impossible, were adsorbed from benzene solution on to the substrate and travelled slowly through the column. Schroeder[30] attributed the higher R_f value for 2-nitrodiphenylamine compared with 4-nitrodiphenylamine to the presence of a chelate bond in the former compound. Le Rosen *et al.*[31,32] provided a similar interpretation for the R_f values of nitroanilines when benzene solutions were used with a silicic acid substrate (Table 3) but they recognised that steric factors were also important. However, when Hoyer[70] applied the adsorption technique to the nitronaphthylamines the results could not be interpreted in terms of intramolecular hydrogen bonding. Isomers such as 5-nitro-1-naphthylamine for which no chelation is possible ran through columns of alumina without adsorption while 2-nitro-1-naphthylamine and 1-nitro-2-naphthylamine, for which others[33,34] have postulated a chelate structure, were adsorbed. It appears that this method for detecting intramolecular hydrogen bonds is still uncertain.

3.6 *Measurement of dipole moment in benzene and dioxan*

The dipole moments of *ortho* and *para* nitroanilines as determined in benzene solution cannot be calculated by vector summation of the moments of aniline and nitrobenzene. There is a strong mesomeric interaction between the groups which results in an increase in the dipole moment. This contribution, the interaction moment, is greatly reduced by groups that are adjacent to the nitro substituent and force it out of the plane of the aromatic nucleus. Smith and Walshaw[35,36] compared the values of the dipole moments of some substituted anilines as determined in benzene and dioxan solutions. They found that values determined in dioxan were considerably higher than those in benzene and that this increase was linearly related to the value of the interaction moment. They attributed the increase to the formation of an intermolecular hydrogen bond, or bonds, to dioxan from the amino group and expected 2,4-dinitroaniline to show a deviation in behaviour because of the presence of an intramolecular hydrogen bond. They found however, that this compound conformed to the general relationship. Their work has been supplemented by Richards and Walker[37] who interpret the evidence of the infra-red spectra as indicative of a single intramolecular hydrogen bond in the molecules of 2-nitro-, 2,4-dinitro-, and 2,4,6-trinitroaniline when in carbon tetrachloride solution, and of a single intermolecular hydrogen bond when dioxan is the solvent. They considered the evidence to be inconclusive as to whether the intramolecular bond was retained in dioxan solution. The change in dipole moment when dioxan is the solvent (Table 4) does not require that more than one hydrogen bond is formed to dioxan but it does indicate that the internal hydrogen bonds are stronger when there are

Table 3 *Effect of substituents on R_f values of phenols and amines**

Compound	R_f	Compound	R_f	Compound	R_f
phenol	0·27	2-nitroaniline	0·48	2-nitrophenol	0·82
aniline	0·21	4-nitroaniline	0·18	4-nitrophenol	0·09
2-anisidine	0·33	2,4-dinitroaniline	0·21	2,4-dinitrophenol	0·62
4-anisidine	0·11	2,4,6-trinitroaniline	0·55	2,4,6-trinitrophenol	0·39

* Values for benzene solutions on a silicic acid substrate[31]

nitro groups in both positions 2 and 6. Their interpretation does not account for a reduction of the measured dipole moment of 2,6-dinitroaniline when it is dissolved in dioxan, or for the observation of Dyall[38] that only the strongest acceptors of hydrogen bonds can produce a change in the infra-red spectra of 2,6-dinitroaniline and 2,4,6-trinitroaniline.

Wepster[39] has shown that a consistent interpretation of the steric effects in the spectra of substituted nitroanilines is possible if the intensity of the absorption band of longest wave length is taken as a measure of the mesomeric interaction between the nitro and the amino groups. Pearson[40] has used this criterion to deduce that the order of mesomeric inter-

Table 4 *Dipole moments and N–H stretching frequencies of nitroanilines*

Substituents	Dipole moments (D)			C_2Cl_4 solution		Dioxan solution	
	$\mu_{benzene}$	μ_{dioxan}	$\Delta\mu$	$\nu_{as}(cm^{-1})$	$\nu_s(cm^{-1})$	$\nu_{as}(cm^{-1})$	$\nu_s(cm^{-1})$
4-nitroaniline	6·22	6·82	0·60	3506	3414		
2-nitroaniline	4·06	4·36	0·30	3519	3398	3466	3346
2,4-dinitroaniline	5·87	6·53	0·66	3518	3392	3463	3324
2,6-dinitroaniline	1·93	1·69	−0·24	3476	3361	3478	3345
2,4,6-trinitroaniline	2·90	3·30	0·40	3457	3344	3444	3312

Richards and Walker also determined the dipole moments in benzene and in dioxan solutions of the four nitronaphthylamines which have the two substituent groups in adjacent positions. They found that $\Delta\mu$ was negligible for 2-nitro-1-naphthylamine and 8-nitro-1-naphthylamine and interpreted this result as indicating no formation of a hydrogen bond to dioxan. This makes it more difficult to account for the negative value of $\Delta\mu$ in 2,6-dinitroaniline and contrasts with the considerable changes in infra-red frequencies which Bryson and Werner[34] attribute to intermolecular hydrogen bonding of these nitronaphthylamines in pyridine solution. The values of $\Delta\mu$ for 1-nitro-2-naphthylamine and 3-nitro-2-naphthylamine were similar to those observed with 2-nitroaniline and were similarly interpreted. It would be useful to have further information on the changes in dipole moments and in infra-red spectrum between the solvents benzene and dioxan for a wider range of primary amines so that a more exact knowledge of the interactions can be gained.

3.7 *Ultra-violet spectra*

The interpretation of an ultra-violet spectrum in terms of the structure of the compound involves inferences regarding the electronic arrangements in both the ground and the excited states. Unless the electronic transition which is responsible for a given absorption band can be exactly identified, all conclusions from changes in frequency or intensity of this band must be taken as tentative.

action for *ortho, meta* and *para* nitroanilines is $p>o>m$ though the infra-red spectra give some grounds for the order $o>p>m$. From the appearance of an extra absorption band in the spectrum of 2-nitroaniline he infers that there is steric hindrance between the adjacent groups. The bathychromic shift of the band at greatest wavelength, when the solvent is changed from cyclohexane to ethanol, is much greater for *para* than for *meta* and *ortho* nitroaniline. This would be consistent with the presence in the *ortho* compound of an intramolecular hydrogen bond which was not broken by solution in ethanol. A similar change in solvent produced smaller bathychromic shifts with 2-nitro-1-naphthylamine and 8-nitro-1-naphthylamine than with other nitro-1-naphthylamines. These are the two nitro-1-naphthylamines for which Bryson and Werner[34] have proposed a chelate structure. Pearson states that 'the nitro-2-naphthylamines form a less satisfactory series' for he found that the bathychromic shifts with 1-nitro-, 3-nitro-, 4-nitro- and 5-nitro-2-naphthylamines are all of similar magnitude.

In an earlier study by Hormann and Endres[41] it was claimed that a hydrogen bond was formed between adjacent groups in 2,4-dinitroaniline, on the evidence that they observed no absorption at the position normally occupied by the long wavelength band of nitroamines when this compound was dissolved in acetic acid. Dyall and Pausacker[42] observed absorption in the relevant region for cyclohexane solutions of 2-nitroaniline and a number of its deriva-

tives. They found that this absorption was intensified in 2,6-dinitroaniline to which all investigators attribute a structure involving an intramolecular hydrogen bond.

4 INFRA-RED SPECTRA OF AROMATIC AMINES

4.1 *General observations on spectra of primary aromatic amines*

It is generally agreed that the study of the infra-red spectrum is, at the present time, the method of choice for the detection of hydrogen bonding. For this reason, infra-red data have been given a special status in the present review. This agreed with the greater concentration of investigations on this approach to the problem.

Aromatic primary and secondary amines have higher N–H stretching frequencies, a reflection of the higher force constants for the bonds, than those for aliphatic amines. The electrons of the 'lone pair' on the nitrogen atom interact with the π bonding system of the aromatic nucleus and the consequent withdrawal of electronic charge causes a change in hybridization of the bonding orbitals of the nitrogen atom from the sp^3 type towards those of the sp^2 type[43]. The N–H bonds will be shorter and stronger as a result of this change but as the withdrawal of electrons has made the nitrogen atom more electronegative the N–H bonds will also have increased polarity. This increased polarity will make the aromatic amine a better donor but a poorer acceptor of hydrogen bonds. In the series — ethane, ethylene, acetylene — we have a similar increase in the electronegativity of the carbon atom and a shortening and strengthening of the C–H bonds. It is the last named, with the strongest and most polar C–H bonds, which acts as a donor in hydrogen bond formation. This change in bond strength with changing hybridization is in the opposite sense to that found with the hydroxyl group. The O–H stretching frequency is lower for phenol in dilute solution in carbon tetrachloride than for methanol and it is still lower in 4-nitrophenol. It is probable that this contrast in behaviour is related to the transition from covalency towards electrovalency as the bond to hydrogen becomes more polar. The difference in electronegativity between oxygen and hydrogen corresponds to 39% of ionic character in the O–H bond according to Pauling's scale (reference 13, p. 98). On the same scale N–H and C–H bonds have 18 and 4% of ionic character respectively. At some degree of ionic character an increase in polarity must result in the potential energy curve showing a broader valley corresponding to the inverse square law for polar attraction instead of the narrow valley characteristic of a strong covalent bond. This could give stronger bonding but lowered frequencies of vibration. If this is so, the low frequencies for the N–H vibrations of 2,4,6-trinitroaniline may, in part, be due to this limiting polarity being exceeded.

When the primary amine forms a unilateral hydrogen bond to an *ortho* substituent, the hydrogen will move away from the nitrogen atom towards the acceptor. This will lengthen and weaken the bond. At the same time the electrons in this bond will move towards the nitrogen atom, reducing its electronegativity and thus the strength of the other N–H bond will fall. If however there is conjugation between the amine group and the *ortho* substituent, as shown for (4) and (5), this excess charge may be largely transferred to the acceptor atom where it will enhance the strength of the original hydrogen bond but will lower the bond order of the C=O or N=O linkage. A fall in the N–H stretching frequencies and also an appreciable decrease in the X=O frequency of an acceptor will therefore be expected.

Where the hydrogen bonds are intermolecular an equilibrium between monomer and hydrogen bonded dimer, or polymer, can usually be studied in dilute solutions with non-polar solvents. For a monomer-dimer equilibrium the sharp, higher frequency, A–H stretching bands of the monomer increase in intensity while the broader, lower frequency, A–H stretching bands of the dimer decrease in intensity as the solution is diluted, in accordance with the equilibrium law. When the bond is formed between two atoms of the same molecule we can have several types of change in the infra-red spectrum depending on the strength of the hydrogen bond in comparison with thermal collision energies, which are of the order of RT(600 cal mole^{-1} at room

temperature). When the bond is weak there will be an equilibrium between the chelated and non-chelated forms as in the classical case of 2-chlorophenol and there will be a doubling of the A–H stretching frequencies. The relative intensities of the absorption bands will not be affected by dilution with non-polar solvents but they will be sensitive to addition of solvents which can act as acceptors of hydrogen bonds and stabilize the non-chelated forms. Dioxan and pyridine have been the solvents used to open such weak intramolecular bonds. If the energy of hydrogen bonds formation is large compared with RT, the absorption at higher frequency, characteristic of the free A–H group, will be absent and only the lower frequency, characteristic of the A–H . . . B system, will be observed in dilute solutions in non-polar solvents. This is the behaviour observed with 2-nitrophenol where theme is a strong, moderately broad, absorption at 3243 cm^{-1} in carbon tetrachloride solution, while there is a sharp absorption band at 3611 cm^{-1} for phenol in the same solvent. When the energy of formation of the bond is much less than RT, the life-time of the bonded form will be short and of the same order as its vibration frequency. In this case no discrete absorption band characteristic of the bonded form will be observed but the A–H stretching frequency may be slightly lowered and the absorption band broadened. It is not possible to distinguish between such weak hydrogen bonding and other perturbing effects of adjacent groups.

The conjugation that can exist between the hydrogen donor and the hydrogen acceptor and between them and the aromatic nucleus can impose a steric situation very favourable to the formation of an intramolecular bond. In formulae (4) and (5) this conjugation energy would add to the energy of any hydrogen bond in resisting the effects of molecular collision. Under these circumstances very weak hydrogen bonds may be maintained but there will be no distinguishable *cis* and *trans* forms of the symmetrical $-NH_2$ group. Rotation of the acetyl group in (5) would be resisted by the steric interaction of the amine and methyl groups. Moritz[8] has invented a way for demonstrating the two arrangements of the amine group in a unilateral hydrogen bond by replacing one hydrogen with deuterium. Under these circumstances the two vibrations of N–H and N–D are not strongly coupled, while hydrogen atoms that are oriented towards, and away from, the acceptor group will have different vibration frequencies. This doubling of the N–H stretching frequency in the –NHD compound provides an exact method of recognising weak hydrogen bonds in this difficult case.

Francel[44] has studied the absorption of polarized infra-red radiation, by oriented films of crystalline 2-nitroaniline. He finds that the intensities of absorptions due to $-NH_2$ and $-NO_2$ stretching motions decrease for changes in the plane of polarization which cause an increase in absorptions due to C–H 'out of plane' vibrations. He treats this as confirmation that the amine and nitro groups are at least approximately coplanar with the benzene ring as required for resonance with the structure (4). On the other hand Trotter[45] claims that the structures of crystalline 2,4,6-trimethyl-1-nitrobenzene and 2,3,5,6-tetramethyl-1-nitrobenzene show that *ortho* methyl groups cause the nitro groups to be rotated out of the plane of the benzene ring. An X-ray diffraction, or better a neutron diffraction, study of the structure of 2-nitroaniline would place the discussion of hydrogen bonding in this and allied compounds on a much firmer basis.

The effect of unilateral hydrogen bonding on the two stretching vibrations of the $-NH_2$ groups will be to uncouple the two motions so that the higher frequency moves towards the mean of the frequencies of the coupled motion. The other vibration will show a much larger displacement to lower frequencies. For strong hydrogen bonds the two motions may be almost completely uncoupled and be characteristic of the free, and bonded, N–H linkages. As most of the acceptor groups are electron-attracting the lowering of the N–H frequencies due to hydrogen bonding may be partially or completely offset by an increase in frequency due to the change in hybridization at the amine nitrogen atom. For this reason the frequencies are best compared with those of the corresponding *para* isomer in which similar conjugative effects can occur but the inductive and direct effects of the *ortho* substituent may be somewhat greater than those in the *para* compound. The situa-

tion is more complex than in the case of the corresponding *N*-alkyl, *N*-aryl and *N*-acyl derivatives of the primary amine. For these only one N–H stretching vibration is expected since the mesomeric effect can bring the two atoms to be bonded into the plane of the aromatic ring and steric interaction between the substituent on the amine nitrogen and the *ortho* group will keep the N–H link *cis* to the acceptor. Under these conditions any interaction which lowers the N–H frequency below the data that Katritzky and Jones[48] obtained with six *ortho* substituted anilines in chloroform solution conform with the above equation. Chloroform is known to act as a donor in hydrogen bonding and it has been found[6] that with simple anilines both the N–H stretching absorptions show pronounced shoulders in this solvent when examined under high resolution. The data are shown in Table 5 together with some values for *ortho* substituted anilines in carbon tetrachloride as solvent.

Table 5 N–H stretching frequencies of ortho*-substituted anilines*

Substituents	$CHCl_3$ Solutions[48]		$CHCl_3$ Solutions[6]		CCl_4 Solutions[6]	
	$\nu_{as}(cm^{-1})$	$\nu_s(cm^{-1})$	$\nu_{as}(cm^{-1})$	$\nu_s(cm^{-1})$	$\nu_{as}(cm^{-1})$	$\nu_s(cm^{-1})$
2-NH_2	3400	3325	3438 < 3425	3352 > 3342	3434	3349
2-OCH_3	3445	3370	3479 < 3456	3390 < 3373	3485	3392
2-Cl	3460	3385	3489 > 3462	3395 > 3377	3489	3395
2-Br.	3450	3370	—	—	—	—
2-C_6H_5	3450	3370	—	—	—	—
2-CH_3	3450	3380	3482 = 3454	3400 > 3381	3482	3396
2-CH_3-4-NO_2	—	—	—	—	3510	3413
2-CH_3-5-NO_2	—	—	—	—	3492	3401
2,4,6-tri-Br.	—	—	—	—	3490	3392

that of the corresponding *para* isomer can be attributed to hydrogen bonding. With the primary amine the differential effects on the two N–H linkages may give a rise in one frequency and a fall in the other. The effect of inductive and mesomeric effects on the $-NH_2$ stretching frequencies is shown as a linear relationship to the Hammett σ factor[46] or as a linear relationship to the pK_a value for the conjugate acid[47]. Bellamy and Williams[10] have shown that for sixty-two *meta* and *para* substituted primary amines the reported values in non-polar solution can be represented by the equation

$$\nu_s = 345{\cdot}5 + 0{\cdot}876\,\nu_{as} \qquad (1)$$

with a standard deviation of 4·8 cm^{-1}. It has been suggested[7,37] that a deviation, greater than 5 cm^{-1}, of the experimental value ν_s from the value calculated from the above equation for an *ortho* substituted amine is evidence of hydrogen bonding. Unfortunately there are too few experimental data to show whether the above relationship holds for *ortho* substituted anilines in which no hydrogen bonding is expected. Richards and Walker[37] consider

Only two of the seven compounds studied in carbon tetrachloride solution showed a good fit with the Bellamy and Williams relationship. Whetsel, Roberson and Krell[49], who have examined a much wider range of *ortho* substituted anilines in the first overtone region of the infra-red spectrum, found that they did not conform to the linear relationship that they had found to exist between $2\nu_{as}$ and $2\nu_s$ for *meta* and *para* substituted isomers. The only cases which did satisfy the relationship had methyl as the sole ortho substituent and this is true of the data in Table 5. However, all the substituents in Table 5, except methyl, have been shown to accept hydrogen bonds from suitable donors.

Since the effect of hydrogen bonding is to lower ν_s strongly and to reduce ν_{as} by a smaller amount, the best presentation of the data will be as a plot of $(\nu_{as} - \nu_s)$ against ν_s. The Bellamy and Williams equation (1) can be rewritten in the form

$$\Delta = (\nu_{as} - \nu_s) = 0{\cdot}1416\,\nu_s - 394{\cdot}4 \qquad (2)$$

which requires Δ to increase as ν_s increases, which is the opposite of the change produced

by hydrogen bonding. If one confines attention to recent results for the N–H frequencies of *meta* and *para* substituted anilines determined at high dispersion[34,47,50], the data shown in Figure 4 for some thirty compounds fit the line,

$$\Delta = (\nu_{as} - \nu_s) = 0{\cdot}4219\ \nu_s - 1348{\cdot}2 \quad (3)$$

found by the method of least squares. The deviations from this line of various *ortho* substituted anilines are shown in the figure.

bush and coworkers[1] were convinced that the lowering of the N–H stretching frequency of 2-nitroacetanilide by some 82 cm^{-1} compared to the corresponding vibration of 4-nitroacetanilide gave definite evidence for the existence of an intramolecular hydrogen bond in the former compound. Dyall and Hambly[3,51], who studied the spectra in C_2Cl_4 solutions of the three mono-nitroanilines and thirteen substituted 1,2-nitroanilines, came to the conclusion that there was no evidence from these spectra for significant intramolecular hydrogen

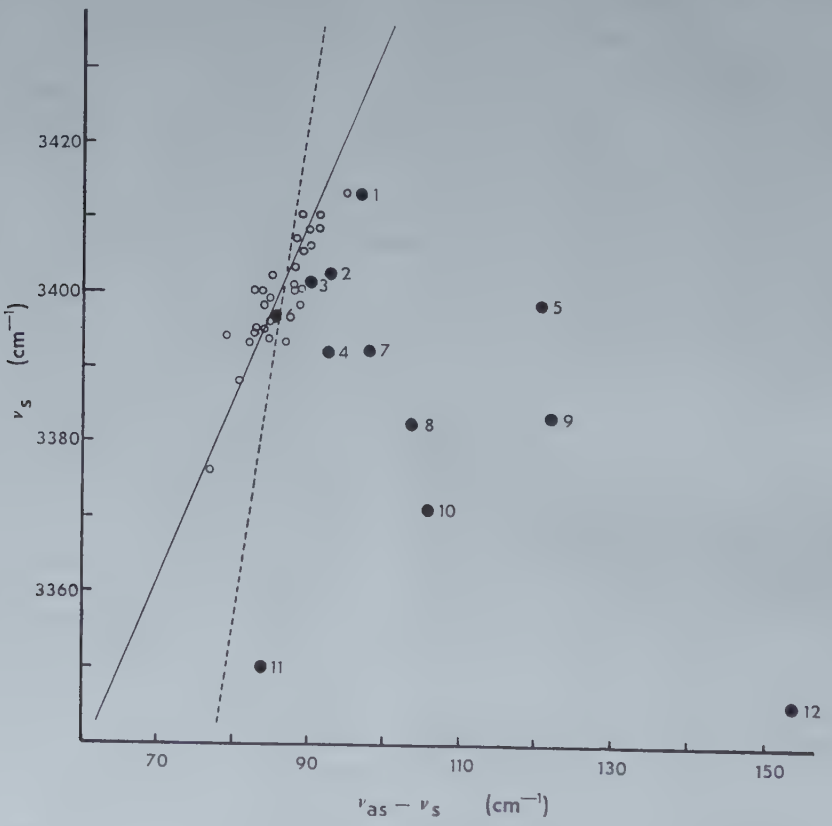


Figure 4 Variation of the N–H stretching frequencies for substituted anilines. Full line, equation (3) *broken line, equation* (2). *Open circles,* meta *and* para *derivatives (references* 47, 50); *filled circles* ortho *derivatives (references* 3, 4, 6). (1) *2-methyl-4-nitroaniline;* (2) *2-chloroaniline;* (3) *2-methyl-5-nitroaniline;* (4) *2-methoxyaniline;* (5) *2-nitroaniline;* (6) *2-methylaniline;* (7) *2,4,6-tribromo-aniline;* (8) *2-amino-diphenylsulphide;* (9) *methyl anthranilate;* (10) *2-aminophenyl, methylsulphone;* (11) ortho *phenylene diamine;* (12) *2-aminoacetophenone*

4.2 *Nitroanilines*

4.2.1 *Fundamental frequencies*

The extent of intramolecular hydrogen bonding in 1,2-nitroanilines has been the subject of considerable discussion in recent years. Rode-

bonding unless there were two nitrogroups *ortho* to the primary amine. These results are in marked contrast to those obtained by Moritz[7,8] with nitro-substituted secondary amines which are also shown in Table 6.

Whereas the mean of ν_{as} and ν_s for the primary amines does not differ appreciably from that of 4-nitroaniline, *ortho* nitration of methylaniline gives a compound with an N–H frequency 34 cm^{-1} lower than that of methyl aniline and 55 cm^{-1} lower than that of 4-nitro-*N*-methylaniline. However, deviations from the values predicted by equation (**3**) are considerable for all the 2-nitroanilines (Table 6). The substituted naphthylamines[28] do not conform to equation (**3**). Substituted 1-naphthylamines all show a smaller value of $(\nu_{as} - \nu_s)$ than is predicted by the equation while most of the substituted 2-naphthylamines show a greater separation (Figure 5). The four nitronaphthylamines with adjacent substituents show extreme departures from equation (**3**) but these were the only naphthylamines studied by Bryson which had the substituent adjacent to the primary amine group.

Table 6 N–H stretching frequencies of nitroanilines

Substituent in Aniline*	ν_{as} (cm^{-1})	ν_s (cm^{-1})	Mean (cm^{-1})	Deviation Equation (**3**)	Substituents in Aniline†	ν (cm^{-1})
—	3482	3395	3428	2·9	N-methyl	3433
4-nitro	3506	3414	3460	—0·2	N-phenyl	3433
3-nitro	3493	3405	3449	—0·4	N-methyl-4-nitro	3454
2-nitro	3520	3399	3460	35·2	N-methyl-2-nitro	3399
2-nitro-6-methoxy	3521	3401	3461	33·3	N-methyl-2-nitro-4-chloro	3401
2-nitro-4-methoxy	3513	3396	3454	32·4	N-methyl-2-nitro-4-bromo	3398
2-nitro-6-methyl	3520	3394	3453	42·3	N-methyl-2-nitro-5-chloro	3394
2-nitro-3-methoxy	3507	3402	3454	17·9	N-phenyl-2-nitro	3355
2-nitro-3-methyl	3505	3402	3454	15·9	N-phenyl-2,4-dinitro	3335
2-nitro-3-chloro	3505	3403	3454	14·5	N-benzyl-2-nitro	3390
2,3-dinitro	3516	3402	3459	26·9	N-acetyl-2-nitro ‡	3370
2,4-dinitro	3518	3392	3455	43·2	N-acetyl-4-nitro ‡	3431
2,6-dinitro	3476	3361	3418	45·2		
2,4,6-trinitro	3457	3344	3400	50·4		

* Solutions in C_2Cl_4 less than 0·05*M*
† Solutions in CCl_4
‡ Solutions in $CHCl_3$

4.2.2 *Solvent effects*

The changes in ν_{as} and ν_s produced by solvents which are recognized acceptors of hydrogen bonds have been quoted as evidence for, and as evidence against, the presence of a chelate hydrogen bond in 2-nitroanilines. Dyall[52] has shown that the variation with changing solvent in the mean frequency $(\nu_{as} + \nu_s)/2$ for 2-nitroaniline bears a linear relationship to the changes produced in the N–H stretching frequency of pyrrole in the same solvent (Figure 6). This shows an extreme contrast to the behaviour of 2-nitrophenol[53]. Even weak acceptors of hydrogen bonds produce appreciable changes in the N–H frequencies of 2-nitroaniline but only more basic solvents such as ether, dioxan or pyridine produce a lowering of these frequencies for 2,6-dinitroaniline and 2,4,6-trinitroaniline[38]. He attributes the difference to the need for a stronger base to break the intramolecular bonds in these compounds. The counter argument is that 2-nitroaniline has at least one N–H linkage not involved in intramolecular bonding and the interaction which Dyall studies may occur through this bond. There could be considerable steric hindrance to intermolecular bonding in the 2,6-substituted aniline.

On the other hand, Bryson and Werner[34] use the change in ν_s and ν_{as} produced by substituting pyridine for carbon tetrachloride as solvent as an indication of intramolecular bonding. This method was originally applied by Short[54] to the detection of chelation in aminoquinolines and aminoacridines. Short argued that if one hydrogen was already involved in chelation there would be less alteration of frequency when the solvent was changed than when both of the N–H bonds were free to form intermolecular bonds to pyridine. The method does not seem to have been tested with a

range of 2-substituted anilines, where no chelation is expected, to assess the effects of steric interaction, but comparisons have been made with isomers where the substituents were further apart. Bryson and Werner appear to have obtained much simpler spectra in pyridine as solvent than were obtained by Dyall[38], Farmer and Thomson[55] or than we obtain with primary

considered this formula to be of doubtful application to any *ortho*-disubstituted aromatic structures and its basis vanishes when one of the atoms is involved in an intramolecular bond. Under these circumstances the 'force constant' k calculated from the equation

$k = 2{\cdot}76_6 \times 10^{-2}\ (\nu_{as}^2 + \nu_s^2)$ dyne cm^{-1}

becomes merely a parameter*. They find k

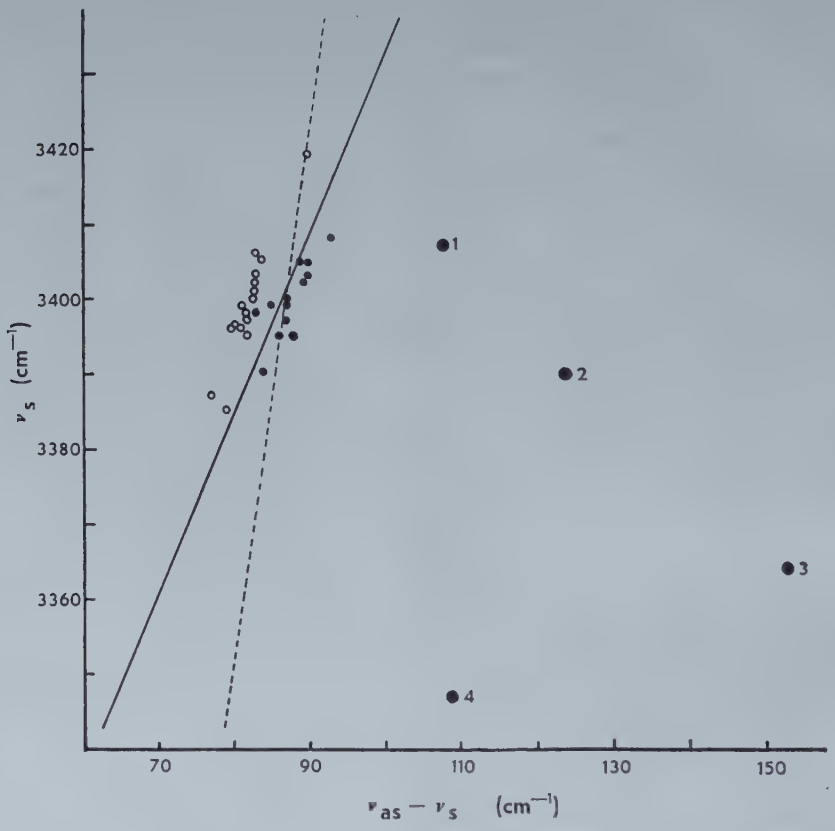


Figure 5 Variation of the N–H stretching frequencies for substituted naphthylamines (reference 28) Full line equation (**3**)*; broken line equation* (**2**)*. Open circles — substituted 1-naphthylamines; small filled circles — substituted 2-naphthylamines; large filled circles — nitronaphthylamines with adjacent substituents.* (1) *3-nitro-2-naphthylamine;* (2) *1-nitro-2-naphthylamine;* (3) *2-nitro-1-naphthylamine;* (4) *8-nitro-1-naphthylamine*

amines in that solvent. In order to obtain a single measure for the effect of changing the solvent they use the formulae of Linnett[56] for calculating the force constant when two identical oscillators are coupled through a common atom, assuming simple valence forces. Linnett

to change with change in solvent by less than $0{\cdot}4 \times 10^{-5}$ dyne cm^{-1} when the nitro and amine groups are adjacent in nitroanilines and nitronaphthylamines, but it changes by more than $0{\cdot}5 \times 10^{-5}$ dyne cm^{-1} when these groups are not adjacent. The calculation of k rather

* There are three misprints in the paper of Bryson and Werner. The equation derived from Linnett's formulae is given as $k = 2{\cdot}76 \times 10^{-5}\ (\nu_{as}^2 + \nu_s^2)$ the ν_s frequency for 1-nitro-2-naphthylamine in CCl_4 should be 3514 cm^{-1}, and k for pyridine solutions of this compound should be 6·26, giving $\Delta k = -0{\cdot}34$.

than the geometric or arithmetic mean of ν_{as} and ν_s does not seem to have significance in this connection.

by Russell and Thompson[57] for secondary amines. The absence of splitting in the fundamental followed by a separation of the order

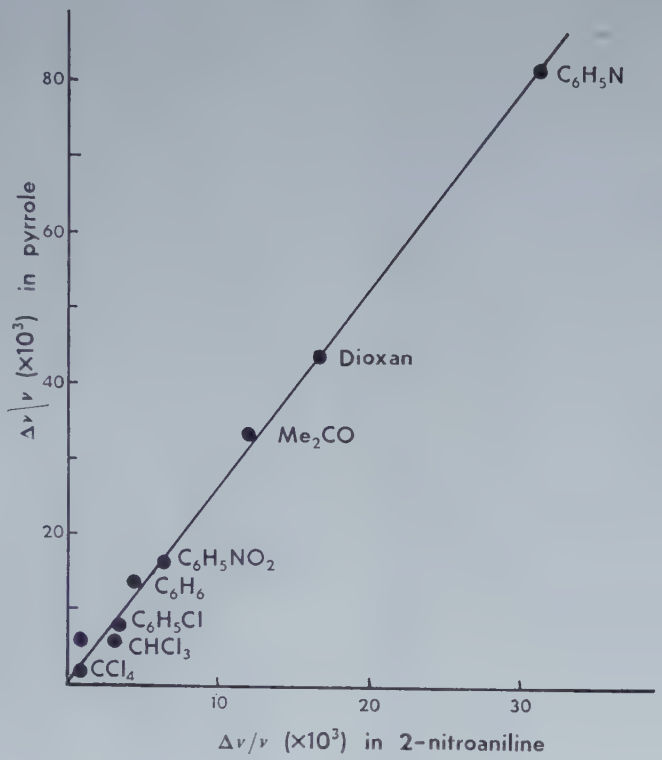
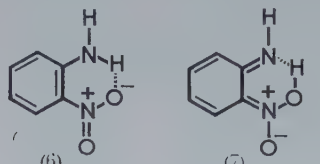


Figure 6 Comparison of solvent effects on N–H frequencies of pyrrole and 2-nitroaniline (reference 52)

4.2.3 *Overtone frequencies*

Whetsel, Roberson and Krell[49] find that the first and second overtones of ν_s for the $-NH_2$ group in 2-nitroaniline are split into doublets but the overtone of ν_{as}, which is of lower intensity, is single. We have confirmed their finding[6] but do not accept their assumption that the higher frequency band of the pair is due to a free N–H linkage while the lower frequency band is due to hydrogen atoms linked by hydrogen bonds to an *ortho* substituent. We find a similar but smaller splitting of the first overtone of the O–H stretching frequency of 2-nitrophenol. The band width at half height for ν_s of 2-nitroaniline is only 34 cm^{-1} in carbon tetrachloride solution and the band is quite symmetrical. If the higher member of $2\nu_s$ were a simple overtone it would show no anharmonicity though the lower member would show a somewhat large anharmonicity factor, $(2{\cdot}83 \times 10^{-2})$, compared with the values found of 100 cm^{-1} in the first overtone is reminiscent of the tunnelling through an energy barrier that Barrow[58,59] has suggested to explain the doubling of some frequencies associated with intermolecular hydrogen bonds. In general there are two positions which the proton can occupy in the bond A–H---B, one closer to the atom A and the other closer to the atom B; for the latter case the bond is best represented as A---H–B. One of these has usually a much lower energy than the other so that there is no splitting of the zero and first vibrational levels and the fundamental frequency is single. There will be some vibrational level at which the energy of the A–H---B system becomes similar to the energy of the ground state for A---H–B, and if the symmetries of the levels are appropriate they will interact and produce a doubled energy level. Barrow deduces that if the pK_a value for the acid A–H, as determined in aqueous solution, is 5 units

greater than that of the acid H–B then there will be a doubling of the fundamental frequency as observed in non-polar solvents. If the pK_a values differ by 15 units then splitting will occur in the second vibrational level and, though the fundamental vibrational transition is single, the first overtone will be doubled. The two structures appropriate to 2-nitroaniline would be (6) and (7). If the suggestion of Barrow is applicable to this case, the doubling of $2\nu_s$ would provide definite evidence for a hydrogen bond in the ground state of

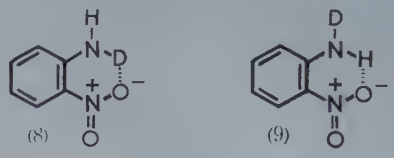


(6) (7)

the molecule. Unfortunately Whetsel *et al.* have not recorded the spectra of any cases such as 2-aminoacetophenone where there is definite evidence for hydrogen bonding from the fundamental frequencies (see section 4.3.2). For methyl anthranilate it has been shown[6] that $2\nu_s$ is a double band but the band of lower frequency is much weaker than that of higher frequency. This shows that the lower band cannot be distinguished as that of the hydrogen-bonded molecule while the upper is for a free N–H group, since the bands are of equal intensity in 2-nitroaniline where the hydrogen bond is known to be much weaker. Recent studies of 2-aminophenyl methyl sulphone[6] lend support to the assumption that the doubling of $2\nu_s$ is an indication of intramolecular hydrogen bonding.

4.2.4 *Partial deuteration*

The most convincing evidence for chelation in 2-nitroaniline is provided by the infra-red spectrum obtained when one of the amino hydrogen atoms is replaced by deuterium. Dyall and Hambly[3] found that the fundamental N–H and N–D stretching frequencies were doubled for 2-nitro-Nd_1-aniline but offered no explanation. Moritz[8] showed that the splitting of the N–H frequency occurred not only in 2-nitroaniline but also when 1-aminoanthraquinone and ethyl anthranilate were monodeuterated. He found also that the difference of the N–H frequencies showed a parallel variation to the strengths of the hydrogen bonds that had been deduced from other evidence. He therefore assumed that the doubling of the absorption band was due to an equilibrium between compounds (8) and (9).



(8) (9)

The separation of the N–H vibrations of (8) and (9) is only 31 cm^{-1} and is of the same order as the splitting due to rotational isomerism in benzylaniline[57] and some anilides[4] and would indicate a very weak hydrogen bond. Where both rotational isomerism and hydrogen bonding would be expected in *N*-benzyl-2-nitroaniline, Moritz finds only a single absorption, showing that the hydrogen bond in secondary amines is sufficiently strong to inhibit the rotational isomerism. The bonding of deuterium to the nitro group in (8) would cause some decrease in the strength of the N–H bond so that the value of 31 cm^{-1} is not a direct measure of the strength of the chelate bond. Short[54] has suggested that when the proton is not in the direct line between the bonded atoms A and B its change of frequency when hydrogen bonds are formed may be much smaller than would occur if the three atoms were colinear. It is doubtful if this applies to the nitroanilines since in crystalline 4-nitroaniline, in which the intermolecular hydrogen bonds are believed to be of the linear type[60], the change in the value of ν_s between the crystal and dilute carbon tetrachloride solution is only 46 cm^{-1}. The value should however be compared with the difference of 65 cm^{-1} between the O–H stretching frequencies of *cis* and *trans* 2-chlorophenol where a hydrogen bond of strength of about 2 kcal $mole^{-1}$ is present in the *cis* form and the difference of 354 cm^{-1} between the O–H stretching frequencies in 2-nitrophenol and 4-nitrophenol[53].

4.2.5 *Nitro group frequencies*

Dyall and Hambly[3] found such a variation in the strong bands in the spectral regions 1530 cm^{-1} and 1350 cm^{-1}, where the antisymmetric and symmetric frequencies of the $-NO_2$ group occur, that they could not interpret these in

terms of conjugative interaction or hydrogen bonding. The variation in the vibration frequencies of the acceptor groups has therefore not provided the evidence for hydrogen bonding that was available from amino-carbonyl interactions.

4.3 *Bonds between amino and carbonyl groups*

4.3.1 *Amino-aldehydes*

Holleck *et al.*[25] published charts of the infra-red spectrum of crystalline 2-aminobenzaldehyde and its dilute solution in carbon tetrachloride. These show that ν_s of the amino group is depressed for the dilute solution to about 3320 cm^{-1} indicating a very strong bond when compared to that inferred for nitroamines. The carbonyl frequency is depressed from 1704 cm^{-1} in benzaldehyde to 1678 cm^{-1} which would suggest that the bond is weaker than that found in 2-aminoacetophenone. They claim to confirm this finding by their polarographic method (See section 3.3).

4.3.2 *Amino-ketones*

Hambly and Bonnyman[4] compared the spectra of 4-aminoacetophenone, 4-aminopropiophenone and 2-aminoacetophenone. The effect of conjugation between the amino and the carbonyl groups was to lower the frequency of the carbonyl absorption below that in acetophenone and to raise the N–H stretching frequencies above those of aniline. In the *ortho* compound this rise in ν_{as} was still found but the symmetric frequency fell from 3408 cm^{-1} in 4-aminoacetophenone to 3343 cm^{-1}, a very significant decrease. This indication of a chelate hydrogen bond of moderate strength is confirmed by the observation that the carbonyl stretching frequency is 26 cm^{-1} lower in the 2-isomer than in 4-aminoacetophenone. The effects of hydrogen bonding are considerably less than those reported by Gordy[61] for 2-hydroxyacetophenone.

4.3.3 *Amino-quinones*

Observations of the spectra of aminoanthraquinones have been mainly confined[46,62] to the crystalline material because of the limited solubility in non-polar solvents. This gives rise to ambiguities of interpretation which depend on the relative strengths of inter- and intramolecular hydrogen bonds. The compound 1-aminoanthraquinone is sufficiently soluble in carbon tetrachloride for the measurement[4] of its amine and carbonyl stretching frequencies. The former show the small depression of the antisymmetric stretching frequency and the larger depression of the symmetric frequency which are expected for a unilateral hydrogen bond. There has been some disagreement in the interpretation of the spectrum in the carbonyl region[46,63,4] arising from a confusion of the amine deformation band with a carbonyl frequency. The spectra can be adequately interpreted by an assignment of bands at 1672 cm^{-1} and 1643 cm^{-1} to the free and the hydrogen bonded carbonyl groups, while the absorption at 1604 cm^{-1} is attributed to the $-NH_2$ deformation mode. The low value for the $-NH_2$ deformation frequency in 1-aminoanthraquinone contrasts with the normal value of 1625 cm^{-1} found for 2-aminoanthraquinone. In general, hydrogen bonding raises deformation frequencies of the bonded atoms because of the extra restraint due to this bond. Moritz[8] has found that the separation of the N–H vibrations of the *cis* and *trans* isomers of monodeuterated 1-aminoanthraquinone is 126 cm^{-1} and the separation between the vibrations, usually designated as ν_{as} and ν_s, for the undeuterated compound is 171 cm^{-1}. These values would indicate the strongest intramolecular hydrogen bonding that has yet been found for aromatic primary amines. They would also suggest that the two N–H groups are becoming sufficiently different so that ν_{as} and ν_s are approaching the stretching frequencies characteristic of the bonded and non-bonded atoms. Under these circumstances we would observe separate N–H deformation frequencies which occur at longer wavelengths than the $-NH_2$ deformation mode (Table 7).

4.3.4 *Amino-esters*

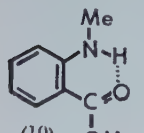
When assessing the effects of hydrogen bonding on infra-red frequencies the choice of the reference compound is important. In one type of reference compound — the *meta* or *para* isomer — intramolecular hydrogen bonding is impossible because of the distance between the substituent groups. The *para* isomer is the better reference because of the similarity in conjugative effects. In the other type of re-

ference compound, hydrogen bonding is eliminated by substituting alkyl groups for hydrogen. These can introduce steric interactions which do not occur in the hydrogen compound.

Table 7 N–H frequencies of monodeuterated primary amines

Compound	$-NH_2$ frequencies (cm^{-1})		N–H frequencies of –NHD (cm^{-1})		Δ(*trans-cis*)
	ν_{as}	ν_s	*trans*	*cis*	
aniline	3478	3395	3430		0
4-nitroaniline	3508	3413	3458		0
ethyl-4-aminobenzoate	3499	3406	3453		0
2-nitroaniline	3519	3398	3477	3446	31
8-amino-2-methylquinoline	3493	3386	3463	3425	39
2-aminophenyl,methylsulphone	3473	3367	3445	3396	49
2-aminodiphenylsulphide	3486	3382	3453	3416	37
methylanthranilate	3505	3377	3478	3412	66
1-aminoanthraquinone	3502	3331	3483	3357	126

When Rasmussen and Brattain[64] compared the spectra of the compounds (10), (11) and (12) they found that the depression of the N–H stretching frequency from 3433 cm^{-1} in methyl aniline to 3361 cm^{-1} for (10) was smaller than they expected from the change in carbonyl stretching frequency from 1727 in (11) and 1732 in (12) to 1685 cm^{-1} for (10). This depression of the carbonyl stretching frequency they believed to indicate a 'chelation of the same magnitude as in salicylates'. On the



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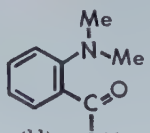

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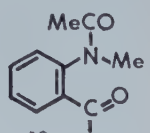


(12)

other hand a comparison of the spectra of ethyl-4-amino benzoate and methyl-2-amino benzoate[4] showed similar values 3499, 3505 for the antisymmetric and 3406, 3383 cm^{-1} for the lower stretching frequencies. These indicate quite a weak interaction which is confirmed by the difference of only 14 cm^{-1} (1711, 1697 cm^{-1}) between the carbonyl absorptions. It is significant that the carbonyl frequencies for the compounds (11) and (12) are considerably higher than those of ethyl-4-aminobenzoate and even higher than that in ethyl benzoate. It appears that steric interaction between the substituted amino group and the ester grouping on the adjacent atom has rotated the latter out of the plane of the aromatic ring. In this position it cannot conjugate with either the π-electron system of the ring or the lone pair electrons of the nitrogen atom. The larger change in the N–H and carbonyl frequencies for (10) compared with methyl anthranilate shows that in this series as with the nitro-anilines the secondary amines form stronger intramolecular hydrogen bonds. In agreement with this conclusion, Farmer and Thomson[9] found that the N–H stretching frequency for ethyl *N*-methylanthranilate is 3370 cm^{-1} in both carbon tetrachloride and pyridine solutions. The separation of the N–H stretching frequencies for the *cis* and *trans* forms of deuterated methyl anthranilate (Table 7) indicates that the strength of the hydrogen bond is greater than in 2-nitroaniline.

The frequencies of the carbonyl stretching motion for ethyl benzoate, acetophenone and anthraquinone are 1722, 1690 and 1676 cm^{-1} respectively. This represents a decreasing bond order in the carbon-oxygen linkage and an increasing negative charge on the oxygen atom. The results discussed above indicate that as the negative charge on oxygen increases, and its electronegativity therefore decreases, the intramolecular bond to a primary amino group increases in strength. The less exact data for 2-aminobenzaldehyde seem to be in agreement with this deduction.

4.4 *Amino-sulphones*

Whetsel *et al.*[49] report the doubling of the overtone bands $2\nu_s$, for 2-aminophenyl-methylsulphone which they believe to indicate hydrogen bonding between the amine and oxygen of the adjacent sulphone group. A comparison[6] of the spectra of (13) (R = methyl, ethyl, isopropyl, or phenyl) with that of (14) indicates the existence of an intramolecular bond in (13) by the changes in the N–H frequencies in the

fundamental region and by the doubling of the $2\nu_s$ overtone frequency. In addition the monodeutero derivative of (13) (R = methyl) shows the presence of *cis* and *trans* forms (Table 7). The values recorded in Tables 7 and 8 indicate that the chelate bonds are stronger in 2-aminosulphones than in 2-nitroanilines. This is surprising in view of the smaller depression of the hydroxyl stretching frequency for 2-hydroxy sulphones compared with the 2-hydroxy nitrobenzenes (Table 9).

Table 8 $-NH_2$ frequencies of aminosulphones and sulphides

Substituent in aniline	Fundamental		Overtones	
	ν_{as} (cm^{-1})	ν_s (cm^{-1})	$2\nu_{as}$ (cm^{-1})	$2\nu_s$ (cm^{-1})
—	3482	3395	6904	6698
4-SO_2-CH_3	3500	3408		
4-SO_2-C_6H_5	3500	3409		
2-SO_2-CH_3	3473	3367	6883	6733, 6628
2-SO_2-C_6H_5	3478	3368	6884	6731, 6627
2-SO_2-*i*-(C_3H_7)	3493 sh.	3367	6898	6744, 6636
	3475			
2-SO_2-C_6H_5	3482	3374	6922*	6729*, 6670*
2-S-C_6H_5	3486	3382	6907	6731, 6658
4-S-CH_3	3481	3394	6905	6698
4-S-C_6H_5	3492	3401	6920	6710

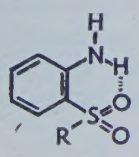
* All data are for very dilute carbon tetrachloride solutions except those marked with an asterisk which were determined in chloroform solution because of very limited solubility in carbon tetrachloride.

Table 9 O–H frequency for phenols in dilute CCl_4 solution

Phenol	4-nitrophenol	2-nitrophenol	2-phenyl sulphonylphenol	2-methyl sulphonylphenol
3611 (cm^{-1})	3597 (cm^{-1})	3243 (cm^{-1})	3317 (cm^{-1})	3324 (cm^{-1})

4.5 *Amino-sulphides*

The absence of hydrogen bonding in hydrogen sulphide[65] which would require sulphur to act both as donor and acceptor, has led to the misconception that sulphur does not take part in the formation of hydrogen bonds. When the sulphur atom is in a thiocarbonyl group it can readily act as an acceptor of a hydrogen bond[67] as is shown by molecular weight determination with thioacetanilide and its *meta* and *para* derivatives. The compounds 2-nitrothioacetanilide and methyl *N*-thioacetoanthranilate do not show association in solution and presumably have a chelate hydrogen bond. M. L. Josien and coworkers[66], who review the earlier literature in which the contrary conclusion was reached, publish data which indicate that thiophenol can dimerize by hydrogen bonding. It can also act as hydrogen donor to a suitable solvent molecule. They were not able to identify intramolecular hydrogen bonding in 2-halogenothiophenols.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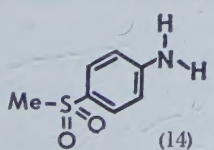
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Whetsel *et al.*[49] have noted a splitting of the first overtone, $2\nu_s$, for the amino group of 2-thiomethoxyaniline, by 107 cm^{-1} and attribute this to hydrogen bonding. Similar doubling of $2\nu_s$ has been noted[6] for 2-aminodiphenylsulphide though the separation is only 73 cm^{-1}. The fundamental $-NH_2$ frequencies for this compound show a deviation of 25 cm^{-1} from the value calculated by equation (**3**). These data can be tentatively interpreted as indicating a weak chelate bond in these compounds. This is confirmed by partial deuteration of the amine group to give *cis* and *trans* isomers (Table 7). The absence of similar interaction in the 2-alkoxyanilines is probably related to the smaller size of the oxygen atom which keeps its lone pair orbitals further from the hydrogen atoms of the amino group. The hydrogen bond in the sulphide would then be

a response to the geometrical conditions rather than to the normal acceptor properties of sulphur atoms.

4.6 Ortho-*diamines*

The formation of a hydrogen bond between two amine groups attached to adjacent atoms would involve the formation of a five-membered ring. Generally the formation of a six-membered ring by hydrogen bonding is more favoured than the formation of a five-membered ring but hydrogen bonds are readily formed by catechol and guaiacol which have structural relationships similar to those in orthophenylene diamine. The fundamental $-NH_2$ stretching frequencies in carbon tetrachloride solution appear as two sharp absorption bands in the infra-red spectrum[6]. This would indicate that the groups are not mechanically coupled and that the structure does not involve an intramolecular hydrogen bond which would be expected to produce four stretching frequencies of N–H linkages.

However the two N–H absorption frequencies in the fundamental region show a departure of 20 cm^{-1} from the prediction of equation (3) and there is a splitting of the overtone $2\nu_s$ by 86 cm^{-1} into two components of unequal intensity. These would be tentative indications of hydrogen bond formation. The prediction that N–H---N bonds will be stronger than those of type N–H---O[68] is not contradicted by the weakness or absence of bonding in *ortho*-diamines and its strength in 2-aminoketones. The difference for these two cases lies in the geometry of the chelate system that is to be formed. No definite bonding can be detected for 2-anisidine which only shows a 10 cm^{-1} departure from equation (3) and no doubling of the overtone $2\nu_s$.

4.7 *Bonding to heterocyclic nitrogen*

The bonding between a substituent $-NH_2$ group and a hetero-atom in the same ring is inhibited by steric considerations though in some cases there is a tautomeric transfer of a proton. When the primary amine group is in the *peri* position of an adjacent ring to the heterocyclic nitrogen, chelation involving the less-favoured five-membered ring structure would be possible. It was to detect weak hydrogen bonds of this sort that Short[54] proposed the use of the change in $-NH_2$ frequencies when the solvent is changed from a non-polar or a proton donor type to a proton acceptor. He concluded from the results set out in Table 10 that there is an intramolecular hydrogen bond in both 1-aminoacridine and 8-aminoquinoline. Moritz has confirmed this conclusion by showing the existence of *cis* and *trans* forms of the monodeuterated 8-amino-2-methyl-quinoline (Table 7). The difference between the *cis* and *trans* forms is of the same order as is found in 2-nitroaniline. The weak hydrogen bond that is indicated by this observation can make important changes in the physical properties of the compound[69].

Table 10. Effects of solvent on N–H stretching frequencies[54]

Compound	Solvent							
	CCl_4		$CHCl_3$		Dioxan		Pyridine	
	ν_{as}(cm^{-1})	ν_s(cm^{-1})	ν_{as}(cm^{-1})	ν_s(cm^{-1})	ν_{as}(cm^{-1})	ν_s(cm^{-1})	ν_{as}(cm^{-1})	ν_s(cm^{-1})
1-aminoacridine	3497	3387	3494	3390	3472	3360	3449	3307
2-aminoacridine	3494	3406	3502	3411	3361	3243	3337	3199
3-aminoacridine	—	—	3494	3400	3367	3241	3337	3206
4-aminoacridine	3474	3394	3477	3399	3363	3260	3351	3218
5-aminoacridine	—	—	3525	3435	3377	3270	3366	3206
2-aminoquinoline			3517	3413			—	—
3-aminoquinoline			3472	3402			3333	3204
4-aminoquinoline			3513	3425			3350	3203
6-aminoquinoline			3488	3402			3338	3210
7-aminoquinoline			3496	3407			3337	3207
8-aminoquinoline			3493	3389			3425	3313

5 CONCLUSION

The evidence presented shows that intramolecular hydrogen bonding of the aromatic

primary amine group to an *ortho* substituent, is very much weaker than in the corresponding phenols. It is even considerably weaker than in the corresponding secondary amines and anilides. In many cases it is only by refined studies of the infra-red spectrum that the existence of intramolecular bonds can be definitely demonstrated. Great caution should be exercised in attributing differences between the properties of *ortho* and *para* substituted anilines to the existence of chelation in the former.

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